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From the Physiological Department, Faculty of Medicine (Karolinska Institutet) and the Surgical Clinic (Karolinska Sjukhuset), Stockholm.

# Adrenaline and Noradrenaline Output in Urine After Unilateral and Bilateral Adrenalectomy in Man

By

U. S. VON EULER, C. FRANKSSON and J. HELLSTRÖM.

Received 22 October, 1953.

The catechol amine output in urine in man is normally about 1/5 adrenaline and 4/5 noradrenaline. This proportion is in good agreement with the relative concentrations recently found in peripheral blood by WEIL-MALHERBE and BONE (1953). The adrenergic nerves in the vessels and in the visceral organs probably represent the most important source of noradrenaline in the urine. Assuming the same concentrations of noradrenaline in human organs and tissues as in the cow or the sheep the total store in the body can be estimated at about 2 mg. Since human suprarenals contain a total amount of about 1 mg noradrenaline (EULER, FRANKSSON and HELLSTRÖM 1954) some of the urinary noradrenaline might be expected to come from this source. Adrenaline, on the other hand, is chiefly derived from the suprarenals (about 6 mg), for the tissues and organs contain only relatively small amounts of adrenaline (0.1—0.2 mg).

In the present report the urinary output of adrenaline and noradrenaline before and after adrenalectomy has been measured in an effort to evaluate the contribution made by the suprarenal glands in the urinary excretion of both catechol amines.

## Material and Methods.

Urine was collected for periods of 24 hours from patients in the surgical ward of the Karolinska Hospital before and various times after adrenalectomy. Part of the urine was also used for 17-ketosteroid analysis (HELLSTRÖM et al., 1952.)

The catechol amines were prepared by adsorption on aluminum hydroxide according to EULER and HELLNER (1951) and the amines assayed biologically on the cat's blood pressure and the fowl's rectal caecum (EULER 1949).

The error of any single figure can be estimated at about $\pm$ 10 % for noradrenaline and about $\pm$ 25 % for adrenaline. No corrections have been made for the loss during the preparation of the extract, which amounts to about 25 %.

Patients selected had cancer of the prostate or breast or malignant hypertension.

Adrenalectomy was performed unilaterally in 2 patients, bilaterally in 2 stages in 4 and bilaterally in 1 stage in 9 patients. Postoperatively the patients were maintained on 25 (—50) mg cortisone per day.

## Results.

The urinary excretion of adrenaline was not altered by unilateral adrenalectomy (table I). However, after total adrenalectomy the adrenaline output is markedly reduced. The excretion figures are so low that the exact excretion rate is difficult to estimate. If, however, the actual excretion rate had fallen to zero a certain number of negative figures should have appeared due to the error in the estimation method. Since this was not the case it is reasonably certain that a small amount of adrenaline is still excreted after total adrenalectomy.

The urinary output of noradrenaline did not change after unilateral adrenalectomy. Although the noradrenaline figures varied a great deal after bilateral adrenalectomy, the highest figures were greater than those before in 10 of 14 cases suggesting an alteration of the noradrenaline output. When the means are compared the significance of the difference is low ($P = > 0.1$). Although high figures of noradrenaline were frequently observed after bilateral adrenalectomy, a lowering of the noradrenaline excretion after adrenalectomy was seldom seen.

## Comment.

Although the disease and post-operative treatment of the patients may have influenced the results in various ways there seems to be little doubt that the adrenaline excretion rate was significantly reduced after complete adrenalectomy. The most plausible explanation for this finding is that the pre-operative

**Table I.**

*Catechol amine output in urine before and after unilateral or bilateral adrenalectomy.*

Average excretion figures in italics. Figures within brackets denote number of estimations over time period in days.

| Subject | Age, sex | Adrenaline excretion μg per 24 hours | | | Noradrenaline excretion μg per 24 hours | | | Diagnosis |
|---|---|---|---|---|---|---|---|---|
| | | Before | After adr.ect. | | Before | After adr.ect. | | |
| | | | unilat. | bilat. | | unilat. | bilat. | |
| A. L. A. .. | 50 F | 8.8 (1/2) | 9.6; 11 *10.3* (2/5) | — | 26 | 27; 29 *28* | — | Ca. mam. |
| S. S. ..... | 38 M | 3.6; 4.8 *4.2* (2/9) | 3.7 (1/3) | — | 44; 64 *54* | 40 | — | Hypertens. |
| B. G. A. .. | 20 M | 3.0—9.8 *5.2* (6/8) | 3.3—9.3 *6.1* (6/16) | 0.78 (1/9) | 9.4—51 *27* | 11—68 *36* | 25 | Hypertens. |
| D. A. E. H. | 37 F | 2.4 (1/1) | 2.1 (1/3) | 0.55 (1/1) | 7.2 | 11 | 22 | Ca. mam. |
| C. M. ..... | 46 F | 5.0—10 *6.9* (3/10) | 2.1 (1/6) | 0.68 (1/8) | 25—26 *26* | 8.9 | 20 | Ca. mam. |
| N. R. N. .. | 45 M | 0—12 *6.4* (6/7) | 1.0—9.9 *6.5* (5/21) | 0—4.0 *1.6* (14/220) | 29—70 *46* | 15—82 *37* | 6—91 *46* | Hypertens. |
| J. E. W. .. | 68 M | — | — | 0—3.6 *1.9* (6/23) | — | — | 25—116 *78* | Ca. prost. |
| N. I. H. .. | 66 M | 2.6—5.1 *3.7* (4/8) | — | 0—1.9 *0.59* (11/190) | 18—35 *28* | — | 1.7—109 *36* | Ca. prost. |
| J. H. ..... | 66 M | 1.6; 3.5 *2.6* (2/14) | — | 0.49—2.3 *1.8* (4/24) | 60; 79 70 | — | 46—197 107 | Ca. prost. |
| E. O. K. .. | 63 F | 5.2; 6.0 *5.6* (2/13) | — | 0.67 (1/3) | 22; 43 *33* | — | 100 | Ca. mam. |
| N. K. N... | 53 M | 0—12 *5.2* (3/5) | — | 0.65—7.7 *4.1* (13/34) 0.2—3.4 *0.7* (5/288) | 30—38 *34* | — | 29—104 *62* 33—77 *60* | Hypertens. |
| K. S. ..... | 60 M | 5.5 (1/5) | — | 0—2.8 *0.80* (12/95) | 26 | — | 7—114 *43* | Ca. prost. |
| K. E. ..... | 64 M | 2.4; 5.0 *2.7* (2/10) | — | 0—4.3 *1.3* (15/44) | 32; 69 *51* | — | 8—135 *64* | Ca. prost. |
| T. O. ..... | 67 M | 3.3—7.8 *5.6* (4/9) | — | 0—3.6 *1.1* (7/95) | 56—113 *72* | — | 25—100 *51* | Ca. prost. |
| E. A. S.... | 55 M | 8.2; 9.7 *9.0* (2/5) | — | 0—2.1 *0.51* (9/95) | 64; 79 *72* | — | 17—132 *52* | Ca. prost. |
| m (average) = | | 5.4±0.52 | 5.1 | 1.0±0.13 | 41±5.2 | 27 | 54±7.4 | |

adrenaline secretion is predominantly derived from the suprarenal medulla. On the other hand there is good evidence for a small persistent secretion of adrenaline even after adrenalectomy. This is most likely due to the production of adrenaline in chromaffin cells outside the suprarenal gland. It should be recalled that most organs contain in addition to noradrenaline small quantities of adrenaline (EULER 1951) and certain amounts may also be present in chromaffin cell groups along the large abdominal vessels.

It is somewhat surprising that the adrenaline secretion is not altered by unilateral adrenalectomy. The number of cases is small, however, and some hypersecretion from the remaining gland during the post-operative period cannot be excluded, especially since post-operative stress often raises the adrenaline output (FRANKSSON, GEMZELL and EULER 1953).

Three of four patients with hypertension were subjected to bilateral adrenalectomy. No particular features as regards the adrenaline or noradrenaline excretion before or after the operation were noted as compared with the other cases. Clinical improvement was noted in two of these patients after a period of several months (EJRUP 1953). In one patient with hypertension (not included in the table) the pattern of catechol excretion before operation differed from the others. On four occasions pre-operative urinary extracts caused only a marked fall of blood pressure in the test cat. This patient died in shock two days after operation. The nature of the depressor agent or the cause of the shock was not established.

In two patients it was observed that the noradrenaline excretion figures gradually diminished shortly before death (7—13 $\mu$g per 24 hours).

An interesting shift in the adrenaline excretion after bilateral adrenalectomy was observed in one patient with hypertension. During the first 5 post-operative weeks the average adrenaline excretion figure was 4.1 $\mu$g per 24 hours which is within the normal range. After this time a rather striking fall in the adrenaline secretion to an average of 0.7 $\mu$g per 24 hours was noted which persisted over a 10-month-period of observation. No definite alteration in the noradrenaline secretion occurred.

It is of some interest to compare the pre-operative catechol excretion figures with those of a group of hospitalized patients with minor ailments. In 21 estimations on seven such patients

the adrenaline output was 4.1 $\mu$g per 24 hours (S. D. = 1.43) and noradrenaline 42 $\mu$g (S. D. = 20), the figures thus showing a good agreement with the pre-operative values reported here.

## Summary.

1. Unilateral adrenalectomy in two patients did not significantly alter the output of adrenaline or nor-adrenaline in the urine.

2. After bilateral adrenalectomy the adrenaline output fell from $5.4 \pm 0.52$ $\mu$g per 24 hours to $1.0 \pm 0.13$ $\mu$g per 24 hours (mean of averages in 14 patients).

3. The output of noradrenaline was frequently increased after bilateral adrenalectomy.

4. The results indicate that most of the adrenaline excreted in the urine is derived from the suprarenals, while noradrenaline comes from other sources, presumably the adrenergic nerves.

*Acknowledgements.* The costs of this study have been defrayed by a grant from the Medical Research Council to one of us (U. S. v. E.). The valuable technical assistance of mrs. A. PURKHOLD and mrs. S. BJÖRKMAN is gratefully acknowledged.

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# Adrenaline and Noradrenaline Content of Surgically Removed Human Suprarenal Glands.

By

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Adrenaline and noradrenaline analyses of human suprarenal glands have been reported by several authors, using biological and colorimetrical methods. The material, however, has chiefly consisted of autopsy material which for several reasons may be unsatisfactory (HÖKFELT 1951, LEMBECK and OBRECHT 1952, SHEPHERD and WEST 1951). In a fraction of a surgically removed fresh human suprarenal the proportion of adrenaline was estimated at 73 % of the total catechols (EULER 1950). Similar or higher figures were obtained by the above mentioned authors. The absolute figures found by HÖKFELT in material consisting of 3 autopsy cases from subjects 45—75 years of age were 0.92—2.3 mg adrenaline and 0.15—0.30 mg noradrenaline per pair of adrenals, while LEMBECK and OBRECHT found in a material of 72 suprarenals mean figures of 0.11 mg per g adrenaline (0.011—0.327) and 0.049 mg per g noradrenaline (0.003—0.19). SHEPHERD and WEST obtained a mean figure of 0.24 mg adrenaline per g in 31 cases.

## Material and methods.

The material used in the present study consisted of surgically removed glands from 9 patients adrenalectomized for cancer of the prostate or the breast and from 4 cases of hypertension.

The glands were brought to the laboratory within 1 hour after removal, and extracted with ethanol acidified with 1.25 ml N-HCl per 100 ml. After grinding with quartz sand the glands were extracted for ½ hour. The filtrate was evaporated to a small volume at low temperature in vacuo and the lipids removed with ether. The ether solution of lipids contains some 5—10 % of the total catechol amines. Correction has not been made for this loss. The clear colourless filtrate was made up to 0.5—1 g per ml with distilled water. Adrenaline and noradrenaline were estimated colorimetrically by the method of EULER and HAMBERG (1949). In a few cases where biological estimation was also made on the cat's blood pressure the results agreed closely with those found colorimetrically.

## Results.

Table I gives the results of the colorimetrical estimations.

**Table I.**

| Subject | Age | Sex | Weight adrenal g | Adr. mg/g | Noradr. mg/g | % Adr. | Diagnosis |
|---|---|---|---|---|---|---|---|
| J. E. W. | 68 | M | 6.0 | 0.34 | 0.085 | 80 | Ca. prost. |
| T. O. | 67 | M | 4.2 | 0.34 | 0.050 | 87 | Ca. prost. |
| N. J. H. | 66 | M | 6.0 | 0.37 | 0.10 | 78 | Ca. prost. |
| K. E. | 64 | M | 5.9 | 0.52 | 0.071 | 88 | Ca. prost. |
| K. S. | 60 | M | 4.8 | 0.58 | 0.10 | 85 | Ca. prost. |
| E. A. S. | 55 | M | 5.5 | 0.42 | 0.060 | 87 | Ca. prost. |
| E. C. I. | 61 | F | 6.8 | 0.62 | 0.16 | 80 | Ca. mam. |
| C. M. | 46 | F | 6.6 | 0.22 | 0.067 | 76 | Ca. mam. |
| D. H. | 37 | F | 4.3 | 0.68 | 0.16 | 81 | Ca. mam. |
| N. K. N. | 53 | M | 5.5 | 0.66 | 0.045 | 94 | Hypertension |
| N. R. N. | 45 | M | 10.5 | 0.27 | 0.044 | 86 | Hypertension |
| K. A. J. | 42 | M | 5.1 | 0.84 | 0.071 | 92 | Hypertension |
| B. G. A. | 20 | M | 5.3 | 0.53 | 0.15 | 78 | Hypertension |
| n = 13 | | M = | 5.9 | 0.49 | 0.090 | 84 | |
| | | | S. D. = | 0.18 | 0.041 | 5.3 | |

The standard deviation is relatively high for both adrenaline and noradrenaline which probably depends partly on the varying amount of cortical tissue present. However, since the material was obtained from patients in various age groups and with serious diseases a closer agreement between the figures might not be expected.

From the figures in the Table it is obvious that adrenaline is the dominant hormone in human suprarenal glands which is in agreement with the findings of previous authors. Thus only an average of 16 % of the total catechol amines was noradrenaline.

From the figures it can be seen that the total amount of adrenaline in a pair of human suprarenals, assuming similar figures in the second gland, is about 6 mg while the total store of noradrenaline is only about 1 mg. When compared with the figures from the cat where the total adrenaline may be estimated at 0.3—0.6 mg and the noradrenaline to about the same figures it follows that the noradrenaline store in human beings is comparatively small.

## Discussion.

It has been demonstrated by HÖKFELT (1951 a) and by SHEPHERD and WEST (1951) that the relative noradrenaline content of the fetal suprarenal is much higher than in the adult organism. The present material does not permit any definite conclusions as to a relationship between the relative adrenaline content and age in adults but no correlation was at any rate obvious. Nor could any marked differences be detected with regard to catechol amine content or percentage adrenaline within the female group (ca. mammae) and the two male groups.

## Summary.

In surgically removed suprarenals from 13 patients with carcinoma or hypertension the average adrenaline content was 0.49 mg per g (S. D. = 0.18) and the noradrenaline content 0.090 mg per g (S. D. = 0.041), the average percentage adrenaline of the total catechol amines being 84 % (S. D. = 5.3).

*Acknowledgements.* The valuable technical assistance of Mrs. A. PURKHOLD and Mrs. S. BJÖRKMAN is gratefully acknowledged. The costs of this investigation have been defrayed by a grant from the Life Insurance Companies' Medical Research Fund.

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# Is the Responsiveness to Epinephrine of the Minute Vessels in the Rat Cecal Mesentery Suitable for Measuring Vasodepressor and Vasoexcitor Material?

By

HELGE BADEN.

Received 2 November 1953.

In 1944 CHAMBERS, ZWEIFACH, LOWENSTEIN and LEE introduced a bioassay for measuring vasodepressor material (VDM) and vasoexcitor material (VEM). They stated, that the minute vessels of the rat's cecal mesentery under certain standardized conditions showed a relatively constant irritability for topically applied epinephrine, and that the irritability changed in a constant way after intravenous injection of VDM and VEM.

This study was undertaken to investigate the constancy of the epinephrine threshold concentration under standardized conditions. While this work was in progress, WIEDEMAN and NICOLL (1953) published the results of a similar study. They found that the epinephrine threshold concentration could be called constant in less than 50 per cent of the rat preparations.

## Method.

Albino rats of unknown origin or Wistar rats weighing 100—125 grams were anesthetized with nembutal intraperitoneally in doses from 30 to 60 mg per kilo body weight. The cecal mesentery was prepared according to ZWEIFACH (1948) and bathed in a Ringer-Gelatin

solution at approximately 38° C. The capillaries were observed through a binocular microscope, and a suitable metarteriole with its true capillaries was chosen for study. With at least 3 minutes interval the drip mechanism was turned aside, and 3—4 drops of epinephrine solution in increasing concentration were applied.

The threshold concentration was defined as the weakest epinephrine solution, which within one minute would effect an unmistakable slowing or a complete arrest of the blood stream in the true capillaries. As soon as the blood stream slowed or stopped, the Ringer-Gelatin drip was restarted. The threshold response was then repeatedly elicited at 3—5 minutes intervals for a period of 1—2 hours, and the necessary concentrations were recorded. The temperature in the drip was checked just prior to each application of epinephrine.

## Results.

The technique was first practised on 32 rats. Of the following 67 rats, only 27 preparations fulfilled the criteria of ZWEIFACH (1948). 13 rats were used to test the reproducibility of threshold responses under basic conditions. In fig. 1 is a graphic representation of the range of variation in the epinephrine threshold concentration. Only in 4 rats was a relatively constant epinephrine threshold concentration obtained, that is, in about a third of the rats. The spontaneous variation in epinephrine sensitivity observed in this study is comparable to the variations recorded in the tables of SHORR and co-workers (1951) and there interpreted as varying degrees of VDM or VEM reactions.

The possibility that variations in threshold after injections of VDM or VEM might exceed the range of spontaneous variation, was investigated on 9 rats. VDM and VEM solutions were prepared from rabbit liver and kidney slices incubated anaerobically in serum, and 0.5 cc were injected intravenously in the test rats. In none of the 9 rats did the variation in epinephrine threshold concentration exceed the greatest variation observed under basic conditions, and in most instances it was well within the spontaneous range of variation.

## Summary and conclusions.

In a study of the constancy of the epinephrine sensitivity of the minute vessels in the cecal mesentery in 13 rats prepared according to ZWEIFACH (1948), only 4 maintained a relatively

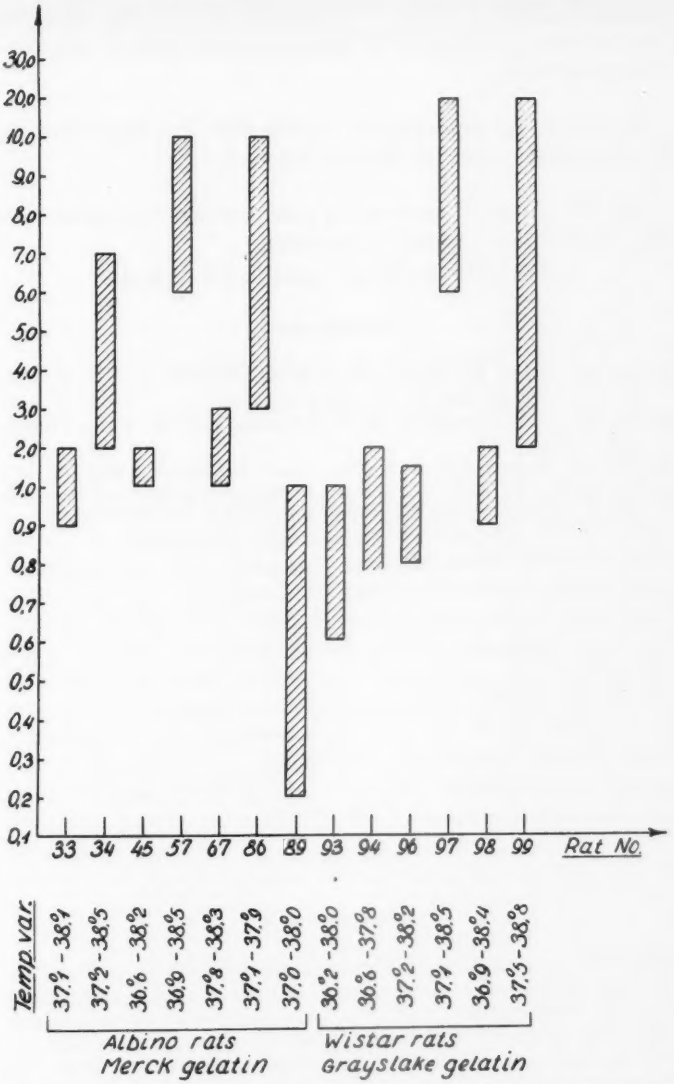


Fig. 1. The columns indicate the variation in epinephrine threshold of the minute vessels in the cecal mesentery of individual rats. The reciprocal value of the figures in the ordinate $\times 10^{-3}$ gives the epinephrine concentration in mg per liter. The abscissa represents individual rats and their serial number. The temperature variation in the drip solution, the rat strain and the Gelatin used in the drip solution are indicated. "Merck" Gelatin contains 1.5 per cent ash, "Grayslake" Gelatin contains 0.25 per cent ash.

constant sensitivity over a 1—2 hours period of study. Thus, the method seems not suitable for measuring vasodepressor and vasoexcitor material.

This work was supported by a grant from The King Christian X's Foundation and by Brandts Legacy.

The "Cyclotherm" used was in part provided by a grant from The General State Science Foundation.

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# Renal Function in Hypothermia.

By

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The influence of a decrease in body temperature has been the subject of extensive experimental work during the last few years mainly in view of providing new means for cardiovascular surgery.

In connection with previously published works from this institution on the present subject the changes of renal functions following a decrease in body temperature and subsequent rewarming have been investigated. Previous research on this problem has been performed mainly on isolated tissues in vitro and has shown a decrease in tubular activity (WINTON and BAYLISS 1948).

A decrease in inulin, creatinine and diodrast clearances at a body temperature of 34—40° in human subjects has been demonstrated by TALBOTT (1951). The extraction ratios were not determined, however.

## Experimental.

Female dogs with a weight between 10 and 22 kg were used in all experiments. Light anesthesia was maintained by intravenous administration of Intraval Sodium. A Pezzer catheter was introduced into the urinary bladder through the urethra or through a small midline opening in the abdominal wall just above the symphysis and into the lumen of the upper part of the urethra. A cannula was inserted into one of the femoral arteries and was connected with an ordinary mercury manometer. Samples of blood could be drawn from the artery through a T-shaped connection with the cannula. A cannula in one of

the femoral veins was used for intravenous infusion and to obtain venous blood.

A catheter was then inserted into the left renal vein. In the early experiments an ordinary heart catheter number 10 F. was introduced through the left jugular vein and through the right aurical into the inferior vena cava. The abdominal wall was opened in the midline and the intestines reflected to the *left*. The inferior vena cava and the proximal part of the left renal vein were thus exposed so that the catheter could easily be directed into the left renal vein with digital aid. In most cases, however, fibrillation of the heart was observed during cooling at a temperature of 25°—22°, probably caused by an irritation of the endocardium by the catheter. The same observation has been made by HEGNAUER et al. (1951).

Thus most of these experiments had to be discarded. A few which could be completed without fibrillation are included in our series since they were identical with the following experiments in all other respects.

In the subsequent experiments a flexible polyethylene tube with an outer diameter of 3 mm and an inner diameter of 1.5 mm was inserted through one of the femoral veins into the inferior vena cava. The abdominal wall was opened in the midline and the left renal vein exposed as above. The tube was then directed into the left renal vein through manipulation with two fingers and was fixed by sutures in the skin of the leg. The tip of the tube was near the hilus of the kidney. The left ovarian vein was ligated.

In a few instances the tip of the catheter slipped into the left adrenal vein for a short time during the operation and in most cases touching of the surface of the left adrenal could not be avoided. The operation could be completed in a short time and without severe loss of blood, and all animals were in excellent condition after the operation.

0.25 g creatinine per kg body weight was given in an intravenous infusion of 1,000—1,500 ml of saline during the operation. A single dose of 1 ml of Umbradil per kg body weight was given intramuscularly, in some cases in connection with a small amount (2—3 ml) intravenously. In two experiments Umbradil was substituted by the same amount of para-aminohippuric acid. Concentrations of the different substances in the blood suitable for clearance determinations were thus obtained for several hours.

The urine was collected during periods of 20—50 minutes duration and in the middle of each period samples of blood were drawn simultaneously from the femoral artery and the left renal vein. The clearances of creatinine and of Umbradil, the tubular excretion of Umbradil and the extraction ratio of Umbradil in the left kidney were calculated during one or several control periods at normal body temperature, and also during cooling to 18°—20° as well as during subsequent rewarming. Cooling and rewarming were performed with water of + 4° and + 40° temperature respectively as described in previous reports. The temperature was measured in the rectum at the end of each period.

During the control period, at the lowest temperature and in some cases at the end of the experiment samples of blood were drawn from

the femoral artery and the left renal vein for analysis of the oxygen content and capacity. The arterio-venous oxygen difference and the oxygen consumption in the left kidney could thus be calculated.

Equal amounts of saline were substituted for blood removed for analyses. The main systolic blood pressure was recorded on the mercury manometer. After cooling had been started no further anesthesia had to be applied. All animals were sacrificed after the experiment.

Colorimetric determinations of creatinine were made by the Jaffe reaction. Double analyses showed a standard deviation of $\pm$ 2.5 per cent.

Umbradil iodine was determined according to ALPERT (1941). The standard deviation has been reported in a previous work (BERGSTRAND 1952).

The oxygen analyses were performed according to VAN SLYKE. Double analyses showed a standard deviation of $\pm$ 0.22 per cent of volume.

The analyses of para-amino-hippuric acid were performed at the clinical laboratory of S:t Eriks Hospital.[1]

## Results.

The results of a typical experiment are given in table 1. The clearance values from all experiments have been classified according to the body temperature at the end of each experimental period. A mean has been computed from all values in each group expressed in ml per min. per kg body weight. The amount of Umbradil iodine, excreted by the tubular epithelium has been computed in the same way as a mean value of all experimental data at each temperature. It is expressed in mg of iodine per min. per kg body weight.

The mean of the clearances and the tubular excretion of iodine have been plotted against the temperatures (fig. 2).

The oxygen content and capacity measured in volume per cent, and the per cent of oxygen saturation in the blood from the femoral artery and the left renal vein are given in tables 2 and 3.

## Discussion.

The clearance of creatinine may be regarded as a true measure of glomerular filtration under the experimental conditions. The main systolic blood pressure decreases with a decrease in temperature and in many cases values are reached at 20° which are close

[1] Head: docent B. JOSEPHSON, to whom we want to express our most sincere thanks for this valuable help.

Table

| Exp. period min. | Temp. °C | Syst. B. P. | Urine | | |
|---|---|---|---|---|---|
| | | | Volume ml/min. | Concentration | |
| | | | | Umbradil-iodine mg/100 ml | Creatinine mg/100 ml |
| 21 | 34 | 120 | 0.76 | 1680 | 1700 |
| 29 | 32 | 115 | 0.61 | 1375 | 1570 |
| 38 | 30 | 120 | 0.30 | 1630 | 2110 |
| 47 | 26.5 | 110 | 0.15 | 758 | 1310 |
| 52 | 22.5 | 100 | 0.14 | 605 | 1120 |
| 46 | 20 | 80 | 0.064 | 764 | 1720 |
| 37 | 19 | 60 | 0 | 0 | 0 |
| 46 | 25 | 85 | 0.046 | 970 | 1980 |
| 32 | 28 | 100 | 0.094 | 1140 | 2320 |
| 35 | 31 | 115 | 0.974 | 1395 | 3800 |
| 34 | 33 | 120 | 0.12 | 1020 | 3540 |
| 16 | 34.5 | 120 | 0.087 | 1400 | 4570 |

to the filtration limit (about 60 m/m of mercury). The excretion of urine may thus decrease considerably and may even be completely abolished. In a few cases no clearence values could be obtained at the lowest temperatures for this reason. It may also be presumed that "delay time" and errors in determination of the excreted volume of urine are important sources of error at the lowest temperatures. The clearance values below 25° of temperature are therefore presumably less exact than those above this limit. These errors can not be of such magnitude that they may have any influence on our conclusions, however.

The clearance of Umbradil is a true measure of renal plasma flow if the substance is completely excreted through the kidney. It is most probable, however, that the extraction ratio (E) of Umbradil is considerably decreased in most pathological conditions. The clearance values will then be proportionally lower than the actual renal plasma flow. A determination of E is therefore necessary for calculations of renal plasma flow during hypothermia. Samples of blood could not be obtained from the renal vein during all periods in our experiments probably due to a decrease in renal blood flow parallel to the decrease in temperature. Thus E could not be determined in more than fifty per cent of all experimental periods. The numbers of observations were none the less sufficient for certain conclusions. Moderate variations

1.

| Plasma concentration | | | Extraction ratio umbradil per cent | Clearance ml per min. per kg body weight | | Tubular excretion Umbradil-iodine mg per kg body-weight per min. |
|---|---|---|---|---|---|---|
| Umbradil-iodine mg/100 ml | | Creatinine mg/100 ml | | | | |
| Artery | Renal vein | Artery | | Umbradil | Creatinine | |
| 9.3 | 6.3 | 26.6 | 32.5 | 30.0 | 3.5 | 0.68 |
| 6.7 | — | 21.3 | — | 19.8 | 3.2 | 0.44 |
| 4.6 | 3.3 | 17.1 | 27.2 | 26.7 | 2.6 | 0.26 |
| 4.9 | 3.1 | 17.3 | 35.8 | 4.6 | 0.8 | 0.052 |
| 4.4 | 3.2 | 17.1 | 28.9 | 4.7 | 0.7 | 0.039 |
| 4.3 | 2.6 | 14.8 | 40.5 | 1.8 | 0.5 | 0.018 |
| 4.4 | — | 15.4 | — | — | — | 0 |
| 4.6 | 2.5 | 17.5 | 45.0 | 1.5 | 0.4 | 0.019 |
| 4.9 | 2.9 | 17.2 | 41.0 | 3.8 | 0.9 | 0.044 |
| 4.1 | 2.5 | 15.2 | 38.8 | 4.8 | 1.3 | 0.036 |
| 3.3 | 2.4 | 12.9 | 28.5 | 9.1 | 2.3 | 0.030 |
| 2.9 | 2.4 | 12.7 | 16.0 | 19.5 | 2.3 | 0.039 |

were observed during the experiments but it could be shown that no regular connection existed between the changes in E and the decrease or rise in temperature. The extraction ratio was practically the same at 20° as at normal temperature.

In the present experiments we are only interested in the *changes* of renal functions evoked by hypothermia. Since no regular changes in E could be demonstrated it may be presumed that this factor is of little importance for our conclusions. This is demonstrated in the diagram (fig. 1). The mean values of Umbradil clearances are plotted against the temperatures. The solid line represents *all* values without correction with E. The dotted line represents these values only which have been corrected with E and thus may be regarded as a true measure of renal plasma flow. The latter figures are too few for certain conclusions per se. The two lines are roughly parallel, however, and in both cases a considerable decrease is observed during hypothermia with subsequent recovery. It may thus be presumed that our values of Umbradil clearance without correction with E are a reasonably true *relative measure* of renal plasma flow under the experimental conditions. The following diagram (fig. 2) is therefore based on these figures.

In most cases the extraction ratio of umbradil and para-aminohippuric acid was considerably lower than the values published

**Table 2.**

| Animal | Temperature | Oxygen | | | | | |
|---|---|---|---|---|---|---|---|
| | | Content | | Capacity | | Saturation | |
| | | A | V | A | V | A | V |
| | normal .... | 13.5 | 11.5 | 14.1 | 14.1 | 96.1 | 81.6 |
| 14 | 18°—20° ... | 14.4 | 10.3 | — | — | — | — |
| | normal .... | 14.7 | 12.7 | 17.8 | 17.8 | 82.6 | 71.6 |
| | normal .... | 12.5 | 10.8 | 13.3 | 13.6 | 94.0 | 79.5 |
| 16 | 18°—20° ... | 16.2 | 15.9 | 18.5 | 19.6 | 87.6 | 81.2 |
| | normal .... | 14.5 | 13.1 | 15.7 | 16.3 | 92.4 | 80.4 |
| 21 | 18°—20° ... | 18.0 | 17.1 | 19.0 | 19.6 | 94.7 | 87.3 |
| | normal .... | 12.6 | 10.8 | 14.3 | 14.3 | 88.2 | 75.0 |
| 23 | 18°—20° ... | 16.5 | 14.6 | 18.0 | 17.9 | 91.6 | 81.5 |
| | normal .... | 12.6 | 10.6 | 16.1 | 15.8 | 78.3 | 67.0 |
| | normal .... | 13.2 | 11.8 | 15.2 | 15.7 | 86.8 | 75.2 |
| 24 | 18°—20° ... | 13.8 | 11.7 | 15.7 | 16.2 | 88.0 | 72.3 |

Oxygen content and oxygen capacity in per cent of volume and oxygen saturation in per cent in arterial (A) and renal venous blood (V).

**Table 3.**

| Temperature | Animal | | | |
|---|---|---|---|---|
| | 14 | 21 | 23 | 24 |
| Normal ..... | 50.8 | 59.9 | 56.9 | 49.6 |
| 18°—20° ..... | 37.7 | 1.8 | 14.4 | 4.0 |

Oxygen consumption in ml $O_2$ per kg body weight per min. in the left kidney.

by PHILLIPS et al. (1946), WHITE (1940) and CORCORAN et al. (1941) in dogs with an explanted kidney.

This may be due to a contamination of blood from the inferior vena cava to the blood that was sucked out through the catheter in the renal vein. This possibility may be ruled out since the difference in oxygen concentration between the arterial and venous blood was very low (table 2).

In a few cases the blood concentration of Umbradil was considerably higher than the ideal value (5 mg per 100 ml. SMITH et al. 1938). This may be of some importance, but it is more probable that the decrease in extraction ratio was mainly caused by the trauma accompanying the operation on the animals.

Two dogs were therefore treated in the same way as previously described. An ordinary heart catheter number 10 F was introduced

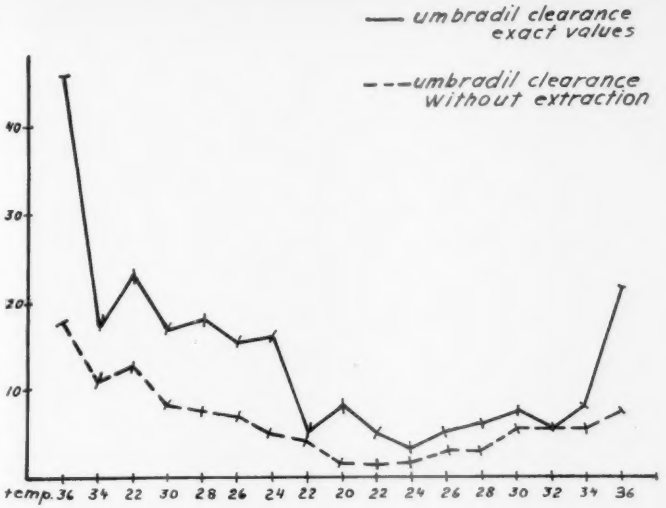


Fig. 1 (see text).

through the left jugular vein, through the right auricle and through the inferior vena cava into the left renal vein without opening the abdominal wall. The position of the catheter was controlled by X-ray. The urinary bladder was not catheterized. During five consecutive periods of ten minutes each at normal body temperature the extraction ratio of Umbradil varied between 47.4 and 54.6 per cent with a mean value of 51.0 per cent in one animal. The arterial blood concentration of Umbradil-iodine was 5.1—2.7 mg per 100 ml. The corresponding values from the second dog were 42.2 and 63.0 per cent with a mean value of 55.0 per cent. The arterial blood concentration was 4.9—3.0 mg per 100 ml. Oxygen analyses of the arterial and renal venous blood showed that there could not have been any appreciable admixture of blood from the inferior vena cava to the samples collected from the renal vein.

It may thus be presumed that the low extraction ratio of Umbradil and para-amino-hippuric acid in our experiments, was mainly due to the influence of anesthesia and probably also of the prone position upon renal function, and that the operative measures were of minor importance.

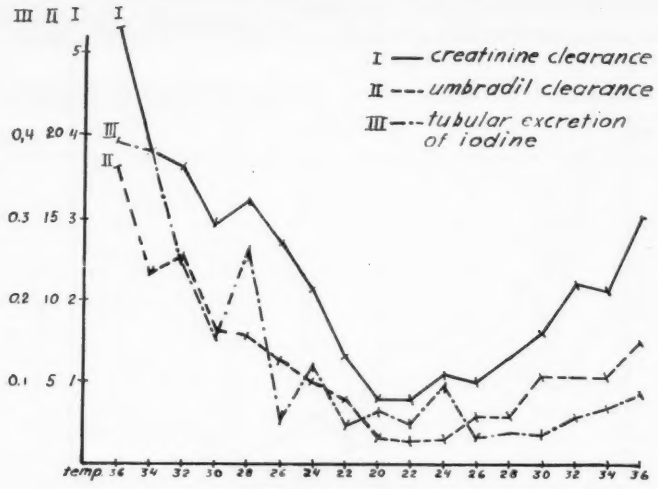


Fig. 2 (see text).

## Conclusions.

The diagram (fig. 2) shows that filtration and renal plasma flow and tubular secretion of Umbradil are decreased during hypothermia. The decrease in renal function is parallel to the decrease in temperature. The changes are reversible and the initial values are nearly reached when the temperature is raised to the normal level. The excretion of Umbradil from the tubular epithelium is slightly decreased at the end of the experiments probably due to a slower recovery of tubular functions. In previous experiments we have made histologic examinations of the kidneys with the routine methods used in this laboratory. We have not been able to demonstrate any changes in the tubular epithelium caused by hypothermia. It is therefore most probable that the tubular functions would also have returned to normal values in a short time in all experiments as was observed in some cases.

The oxygen consumption in the left kidney was measured successfully in only four animals. It was considerably decreased during hypothermia. The differences in oxygen content and oxygen saturation between the arterial and renal venous blood were considerably altered in some experiments, but no regular changes were observed.

## Summary.

Filtration and renal plasma flow are measured by creatinine and Umbradil clearance in dogs at normal temperature during a decrease in body temperature to 18°—20° and during rewarming. The tubular excretion of Umbradil is also calculated. Changes in renal functions parallel to the decrease and increase of temperature are observed. The oxygen consumption in the kidneys is considerably decreased at low temperatures but the differences in oxygen content and saturation between the arterial and renal venous blood shows no regular alterations.


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# Stimulation of Thyroid by Exogenous Thyrotropic Hormone in Young Guinea Pigs Subjected to Subtotal Thymectomy.

By

L. GYLLENSTEN and A. OLSSON.

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## Introduction.

It has been demonstrated that subtotal thymectomy in young guinea pigs induces a slight hyperactivity of the thyroid gland (GYLLENSTEN 1953). Fourteen days after the operation a slight hyperplasia of the lymphatic tissue was found. The hyperplasia of the lymphatic tissue could be explained by the action of the increased secretion of the thyroid hormone. Four weeks after the operation no statistically significant morphological stimulation of the thyroid could be demonstrated. According to RAWSON et al. (1949, 1952) explants of thymus and lymphnodes from rabbit were found capable of inactivating thyrotropic hormone. It might thus be suggested that the effects mentioned above are due to a similar capacity of the same tissues *in vivo*. The subtotal thymectomy may intensify the action of endogenous thyrotropic hormone, the resulting hyperthyroidism produces a compensatory lymphatic hyperplasia, and the increased amount of lymphatic tissue inactivates the excess of endogenous thyrotropic hormone. Thus, a balance of thyroid activity will result, the pituitary thyrotropic hormone and the thymo-lymphatic tissue acting as regulators.

An apparent support of this view was found in the increased action of exogenous thyrotropic hormone administered to guinea pigs subjected to subtotal thymus resection five days previous to the hormone injections (GYLLENSTEN 1953). 1 Ms E thyrotropic

hormone (according to the definition by HEYL and LAQUEUR 1934/35) daily during three days was injected intraperitoneally on the fifth, sixth and seventh days after the operations. The animals were killed on the eighth day, 24 hours after the last injection, and their thyroids judged as to activity by their weights and mean acinar cell heights. As controls were used animals injected with thyrotropic hormone but not subjected to thymus resection, animals subjected to operation but not treated with hormone, and animals neither operated on nor treated with hormone. The animals with a reduced thymus mass showed a considerably greater thyroid activation after treatment with thyrotropic hormone than the normal ones, and this increasement of the stimulation was greater than the slight stimulation induced by the mere thymus resection. Thus the operative reducement of the thymus tissue caused an augmentation of the thyroid reaction to thyrotropic hormone.

These findings are consistent with the theory that the thymus *in vivo* has an inactivating action on thyrotropic hormone. At about one week after the thymus resection the postoperative thyroid activation was found to be maximal, as judged by oxygen consumption of the animals (GYLLENSTEN 1953). Presumably the compensatory lymphatic hyperplasia has not yet occurred at the time of the injections mentioned above. At about two weeks after the operation, however, the thyroid hyperactivity began to decrease, and at the same time the lymphatic hyperplasia was found.

These results do not necessarily mean a confirmation of the hypothesis that the thymus *in vivo* inactivates the thyrotropic hormone. Other explanations of the results are possible (for discussion, see GYLLENSTEN 1953).

The theory of Rawson as to the specifically inactivating action of thymus and lymphatic tissue on thyrotropic hormone would find a support if it could be shown that the increased action of thyrotropic hormone in animals subjected to thymus resection would diminish later on, when the compensatory lymphatic hyperplasia is established. This is the problem of the present investigation.

## Methods.

Three groups of animals (young guinea pigs) were used. The animals of the first group (26 animals) were operated on by

subtotal thymus extirpation, approximately 97 per cent of the thymus tissue being removed. During the fifteenth to seventeenth days after the operation the animals were subjected to intraperitoneal injections of thyrotropic hormone, 1 Ms E daily (according to the definition by HEYL and LAQUEUR). The hormone preparation used was Ambinon (Pharmacia), which is a pituitary extract from cows. The hormon was dissolved in Ringer's solution, so that 1 ml contained 2 MsE. The animals were killed on the eighteenth day after the operations, 24 hours after the last injection. The animals were killed by a blow on the back of the skull, and their thyroids were carefully dissected and weighed. After that the thyroids were fixed in 10 % formalin for 24 hours, embedded and sectioned in five microns thick sections. The sections were stained with iron-alum-hematoxylin (HÄGGQVIST). 50 acinar cells from the thyroid lobes of each animal were measured after projection on the focusing screen in a microcamera at a magnification of 1000. The technique is described in detail by GYLLENSTEN (1953).

The animals of the second group (24 animals) were operated on in the same manner. The injections were performed as previously mentioned but on the fifth, sixth and seventh days after the operation. The animals were killed on the eighth day and their thyroids prepared as the thyroids of the animals in the first group.

The guinea pigs of the third group (24 animals) were not subjected to any operation. They were given hormone injections for three days exactly as the animals of the first two groups, and their thyroids were prepared in the same way.

The animals of these three groups were treated concomitantly. The hormone solutions used during the days of injections were the same for the whole period of injections and for the animals of the different three groups. As described by GYLLENSTEN (1953) no differences between the sexes in the reactions studied are to be expected, and no such differences were found in the present material. For that reason no specifications of the animals as to sex will be presented.

## Results.

The correlations between thyroid weights and body weights in the three groups of animals are presented in Figure 1. The quantitative data are apparent from Table 1.

The differences in body weight between the groups are statistically non-significant. Because of the small coefficients of regression these very small body weight differences have no bearings on the difference between the thyroid weights of the three groups. The mean acinar cell heights are not influenced by the body weights.

The increase in thyroid weight and mean acinar cell height

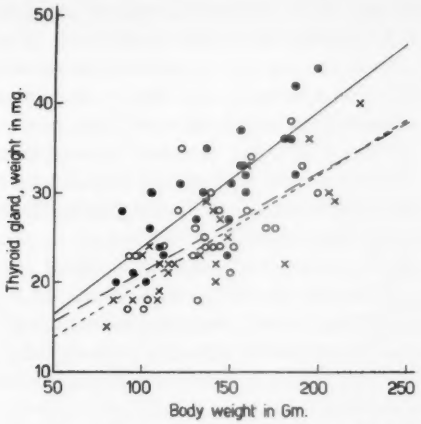


Figure 1. Correlation between thyroid weight and body weight in guinea pigs subjected to subtotal thymus extirpation 5 days before treatment with thyrotropic hormone (●, regression line ———), 15 days before the same treatment (○, regression line — — — —) and in guinea pigs not subjected to any operation before the hormone injections (x, regression line - - - -).

**Table I.**

*Mean thyroid weights, mean body weights, and mean acinar cell heights in animals subjected to subtotal thymus extirpation 15 days previous to the beginning of the injections of thyrotropic hormone (Group 1), in animals subjected to operation 5 days before the beginning of the injections (Group 2), and in animals not subjected to any thymus resection before the injections (Group 3). The figures are approximated as regards the last decimal, which is the reason why they do not exactly correspond to the figures used in the calculations later on.*

| Animals | (Y = thyroid weight, mg X = body weight, Gm) Line of regression | (mg) Mean thyroid weight | (Gm) Mean body weight | (microns) Mean acinar cell height | Number |
|---|---|---|---|---|---|
| Group 1 | Y = 0.114 X + 9.85 | 25.65 | 138.6 | 6.057 | 26 |
| Group 2 | Y = 0.152 X + 8.53 | 29.92 | 138.8 | 6.718 | 24 |
| Group 3 | Y = 0.122 X + 7.67 | 24.42 | 137.3 | 6.158 | 24 |

after injection of thyrotropic hormone is greater in animals subjected to subtotal thymectomy five days before the beginning

of the injections (Group 2) than in normal animals (Group 3). The difference in increase of thyroid weight is 5.50 mg, the standard error of this difference is 1.81, which corresponds to a t-value of 3.039 and a p-value between 0.01 and 0.001. The difference in increase of mean acinar cell height is 0.559 microns, standard error 0.179, t-value 3.124 and p-value between 0.01 and 0.001. Both these differences are statistically significant according to the conventions of BONNIER and TEDIN (1940). These results are similar to those of GYLLENSTEN (1953).

The animals of group 1, subjected to subtotal thymectomy 15 days before the beginning of the injections, did not show any augmentation of the thyroid reactions to stimulation by thyrotropic hormone, as compared with the animals subjected to hormone injections but not to any previous thymus resection (Group 3). The mean thyroid weight of the animals of group 1 is slightly greater than the mean thyroid weight of the controls of group 3, but this difference is statistically insignificant (difference 1.24 mg, standard error 1.60, t-value 0.773). The mean acinar cell height of group 1 is less than that of group 3, but this difference is also statistically insignificant (difference 0.101 microns, standard error 0.172, t-value 0.587).

The previous calculations demonstrate that the augmentation of thyroid sensitivity to stimulation by thyrotropic hormone, which is apparent five to seven days after subtotal thymectomy, is not demonstrable 15 to 17 days after the operation. This difference between the animals of group 2 and group 1 is statistically highly significant. The difference in mean thyroid weight between the animals of group 2 and 1 is 4.26. The standard error of this difference is 1.66, which corresponds to a t-value of 2.573 and a p-value between 0.02 and 0.01. This difference is statistically almost significant, according to the previously mentioned conventions. The difference between the groups as regards mean acinar cell height is 0.660 microns, with a standard error of 0.175, corresponding to a t-value of 3.773 and a p-value of less than 0.001. This difference is statistically highly significant.

## Summary.

1) The increase of thyroid weight and mean acinar cell height after stimulation with thyrotropic hormone was studied in young guinea pigs subjected to subtotal thymus resection. If the opera-

tion was performed five days before the beginning of the stimulation an increased reaction of the thyroid was found. If the operation was performed 15 days before the beginning of the injections no such increasement could be demonstrated. The difference in reaction between these two groups of animals was statistically highly significant.

2) These results may be related to the reports of RAWSON et al. (1942, 1949, 1952) as to the inactivating effect of thymus and lymphatic tissue on thyrotropic hormone, and to the observations of GYLLENSTEN (1953) as to the hyperplasia of lymphatic tissue demonstrable two weeks after subtotal thymectomy in young guinea pigs. The thyroid hypersensitivity shortly after subtotal thymectomy may possibly later on be lost, due to a compensatory action of the hyperplastic lymphatic tissue.

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# The Effect of the Carbonic Anhydrase Inhibitor, Diamox, on Urine Electrolytes in Normal and Adrenalectomized Dogs, and its Possible Relationship to Serum Bicarbonate.[1]

By

POUL OVE MADSEN.[2]

Received November 30, 1953.

The existence of an enzyme, carbonic anhydrase, catalyzing hydration and dehydration of carbon dioxide was definitely proven in 1932, (BRINKMAN, MARGARIA, MELDRUM and ROUGHTON 1931, 1932 VAN SLYKE and HAWKINS 1930). Since then further investigations have dealt with the localization of this enzyme in the tissues. It has been found in high concentration in erythrocytes, in the gastric mucosa, in the pancreas, and in the renal cortex. To various extent it has been found in many other animal tissues (DAVENPORT 1939, 1946).

Further work on the enzyme included the study of its inhibitors. Among nonspecific inhibitors are heavy metals (MELDRUM and ROUGHTON 1933), oxidizing agents (KIESE and HASTINGS 1940), sulfides, cyanides, azides (DAVENPORT 1940, FELDBERG, KEILIN and MANN 1940), and negative ions (KIESE and HASTINGS 1940).

Sulfanilamide is a specific inhibitor of the carbonic anhydrase and the inhibiting action was found to be due to a free-$SO_2NH_2$ group (MELDRUM and ROUGHTON 1933, MANN and KEILIN 1940, DAVENPORT 1945). When used in the clinic, sulfanilamide

[1] This investigation was supported with funds from the Dr. HENRY C. BUSWELL Memorial.

[2] Fellow in Urology Research.

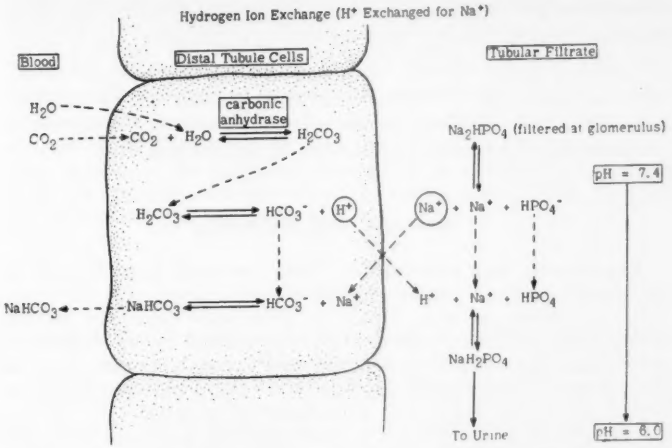


Fig. 1. Carbonic anhydrase catalyzes the hydration of $CO_2$ to carbonic acid. The hydrogen ions formed by dissociation of this carbonic acid are exchanged for sodium (and potassium) ions. Diamox paralyzes the carbonic anhydrase and thus no hydrogen ions will be available for the exchange for sodium (and potassium). (Courtesy of H. A. HARPER (35). University Medical Publishers, Palo Alto, California.)

often caused a drop in plasma bicarbonate, a rise in urinary pH and an increased urinary excretion of sodium, potassium and water (SOUTHWORTH 1937, STRAUSS and SOUTHWORTH 1938). Recently a much more potent specific carbonic anhydrase inhibitor has been found, Diamox, (2-acetylamine-1, 3, 4 thiadiazole-5 sulfonamide)[1] (KIESE and HASTINGS, MILLER, DESSERT and ROBLIN 1950). Its structure and pharmacological activities are completely different from the bacteriostatic sulfonamides.

The proposed action of carbonic anhydrase in the acidification mechanism of the kidney is illustrated in figure 1. In recent works with Diamox in dogs (BERLINER and collaborators 1950—1952, MAREN 1952) as well as in humans (FRIEDBERG and coll. 1952, 1953, BELSKY 1953) it was noticed that with prolonged treatment the effect on urine flow and electrolyte excretion decreased and finally disappeared altogether. Also a pronounced drop in plasma bicarbonate was observed (MAREN, WADSWORTH and YALE).

The present study was carried out to evaluate if the lack of

[1] Developed by Laboratories of the Chemotherapy Division of American Cyanamid Company.

effect after prolonged treatment could be explained by the simultaneous drop in plasma bicarbonate concentration. As it is still unknown how the adrenal cortical hormones affect the electrolyte reabsorption in the kidneys, an attempt to elucidate this problem was made by evaluating the effect of Diamox in the adrenalectomized dog compared to the effect in the normal dog.

## Material and Methods.

Experiments were performed on female mongrel dogs. One to two experiments were performed on each of seven normal dogs and one to two experiments on each of three of these same dogs after bilateral adrenalectomy. The adrenalectomy was performed in two stages at an interval of one week. The adrenalectomized dogs were maintained on daily doses of intramuscular injections of 2.5 mg of desoxycorticosterone acetate (Cortate)[1] and 25 mg of cortisone (Cortogen)[1].

For three days preceding the experiment the hormones were withdrawn. The three adrenalectomized dogs were kept alive for four to six weeks after the experiments and were then sacrificed by withdrawing the hormones. Their blood chemistry after withdrawal of the hormones showed typical signs of adrenal insufficiency and at autopsy no residual cortical tissue was found.

In order to induce acidosis and alkalosis, four dogs were given ammonium chloride and one sodium bicarbonate respectively.

In most of the experiments the following standard procedure was used: Under intravenous nembutal anesthesia, 3 cc/kg of an 8 % creatinine solution was given subcutaneously. This was sufficient to maintain a serum creatinine level of 10 to 15 mg % throughout the experiment. In order to obtain a sufficient diuresis for the clearance determinations, an infusion of 5 % dextrose in water (10 cc/kg/hour) was given for approximately one hour prior to the clearance periods to the end of the experiment. Creatinine clearance was determined in three successive 20—25 minute periods before and after the intravenous injection of 10 mg/kg of Diamox[2] dissolved in a 2 % sodium hydroxide solution. The urine was collected under oil and the urinary pH was recorded continuously in a closed system (Schlegel 1952). The blood pressure in the femoral artery was recorded using a Statham pressure transducer. Blood samples for determination of creatinine, sodium, potassium, chloride and bicarbonate were drawn 1—3 minutes before the middle of each clearance period. The bladder was emptied by suprapubic pressure at the end of each clearance period. The bladder was not rinsed since the clearance periods were in successive order. Plasma and urine sodium and potassium determinations were per-

[1] Furnished through the courtesy of Schering Corporation, Bloomfield, New Jersey.

[2] Furnished through the courtesy of Lederle Laboratories, Pearl River, New York.

formed on a Barclay flame photometer with internal lithium standard. Chlorides were determined by the method of Sendroy and modified by VAN SLYKE and HILLER (1947). Ammonia content of the urine was determined by the aeration method of VAN SLYKE and CULLEN, modified by SUMMERSON (1947) and total $CO_2$ in plasma and urine by the method of VAN SLYKE and NEILL (1927). Calcium in urine was determined by a modified Kramer-Tisdall method (1921), and phosphate in urine by the method of FISKE and SUBBAROW (1925). Creatinine in plasma and urine was determined by the method of Jaffe, modified by PETERS (1942).

## Results.

Table 1 shows some of the data from a characteristic experiment in a normal dog. Since Diamox prevents the reabsorption of cations in the kidney tubules the effect of Diamox will be expressed as the increase in sodium plus potassium excretion per kg/min. In figure 2 the effect of Diamox in fourteen of the experiments is plotted against the plasma bicarbonate concentration prior to the injection of the inhibitor. A relationship between the plasma bicarbonate concentration and the effect on the urinary excretion of sodium and potassium seems obvious. Furthermore it can be seen that the effect of Diamox is not different in the normal and the adrenalectomized dogs.

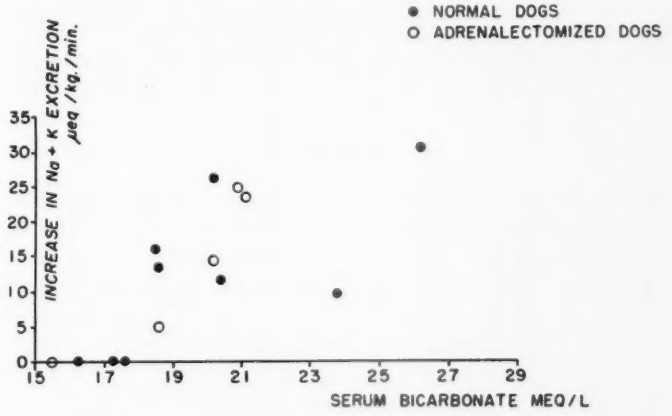


Fig. 2. The relationship between the effect of Diamox on the urinary excretion of sodium and potassium and the serum bicarbonate concentration prior to the injection of the inhibitor.

**Table I.**

*Dog no. 14. Weight 20 kg (normal dog).*

The filtered amounts have been calculated from the glomerular filtration rate (creatinine clearance) and the plasma concentration multiplied by 1.05 for $Cl^-$ and $HCO_3^-$ and 0.95 for Na and K. Water content of plasma is 94 %.

| Time min. | Urine flow cc/min. | Urine pH | Creatinine clearance cc/min. | Plasma (Meq/l) | | | | Excretion rate ($\mu$eq/min.) | | | | | | | Reabsorbed In % of Filtered Amount | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Na | K | Cl | $HCO_3$ | Na | K | Ca | $NH_3$ | Cl | $HCO_3$ | $PO_4$ | Na | K | Cl | $HCO_3$ | $H_2O$ |
| 0—22... | 2.21 | 5.50 | 92.3 | 138.4 | 4.60 | 110.0 | 19.6 | 73 | 5 | 2 | 15 | 82 | 4 | 24 | 99.4 | 98.8 | 99.2 | 99.8 | 97.5 |
| 22—44... | 1.66 | 5.70 | 91.9 | 139.4 | 4.32 | 111.0 | 18.9 | 45 | 8 | — | 21 | 68 | 1 | 41 | 99.6 | 97.8 | 99.4 | 99.9 | 98.0 |
| 44—66... | 2.09 | 5.80 | 85.6 | 133.4 | 4.00 | 110.5 | 17.9 | 15 | 5 | 2 | 19 | 38 | 1 | 15 | 99.8 | 98.5 | 99.6 | 99.9 | 97.4 |
| | | | | Intravenous injection Diamox 10 mg/kg | | | | | | | | | | | | | | | |
| 66— 88.. | 4.21 | 7.40 | 78.6 | 138.6 | 5.02 | 107.0 | 18.4 | 275 | 167 | 4 | 6 | 52 | 305 | 40 | 97.3 | 55.5 | 99.4 | 79.9 | 94.3 |
| 88—110.. | 4.57 | 7.35 | 64.0 | 139.2 | 4.85 | 112.5 | 18.4 | 240 | 89 | 1 | 1 | 26 | 250 | 72 | 97.2 | 69.8 | 99.7 | 79.8 | 92.4 |
| 110—132.. | 4.09 | 7.35 | 63.2 | 137.4 | 5.17 | 107.0 | 17.5 | 212 | 99 | 1 | 1 | 19 | 165 | 86 | 97.4 | 68.1 | 99.7 | 85.8 | 93.1 |

No relationship between the effect of Diamox and the sodium, potassium or chloride concentration in the plasma could be demonstrated.

In none of the experiments was there any significant change in the calcium and chloride excretion in the urine. Phosphate excretion increased in one experiment (Table 1), decreased in two, and was unchanged in four experiments. In all the experiments there was a decrease in ammonia excretion. The effect of the drug is instantaneous and from the continuous pH recording a sudden increase in urinary pH could be seen 1—2 minutes after the injection in all experiments.

Blood pressure and creatinine clearance were determined simultaneously in three of the experiments and a 10—15 mm Hg. drop in blood pressure which started 5—10 minutes after the injection of the inhibitor and which lasted for approximately an hour was recorded simultaneously with a 10—15 % drop in glomerular filtration rate.

In eight out of the fourteen experiments where creatinine clearance was determined, the injection of 10 mg/kg of the inhibitor caused a significant fall in glomerular filtration rate (the average fall was approximately 15 %). From this it seems reasonable to assume that the drop in glomerular filtration rate which is occasionally found, is caused by a slight drop in the blood pressure.

No serious effects of Diamox was observed in any of the dogs. Pulse rate and respiration rate were unchanged after the injection of the inhibitor.

## Discussion.

Prolonged administration of Diamox (every 6—8 hours) has been found to lead to frank acidosis (Maren, Wadsworth and Yale). It has further been observed that the drug failed to influence the water and electrolyte excretion after prolonged administration.

This study indicates that a low serum bicarbonate will minimize or nullify the effect of Diamox on urine flow and electrolyte excretion. It therefore seems reasonable to assume that the lack of effect of Diamox after prolonged administration is a consequence of the resultant lowering of serum bicarbonate. When Diamox is discontinued for 24 hours it will again have its usual

effect probably because the organism has recovered from the acidosis. It was observed that Diamox had no effect in a patient when the serum bicarbonate was lowered as a result of a metabolic acidosis.

These observations seem to suggest that Diamox will have little or no effect in certain conditions with acidosis.

That the effect of Diamox in the adrenalectomized dog is the same as in the normal probably means that the physiological function of carbonic anhydrase in the kidney is independent of the adrenal hormones. This together with the instantaneous effect of Diamox on the urine electrolytes, seems to indicate that Diamox very likely has a direct effect on the kidney.

The lack of effect of Diamox on calcium excretion probably means that calcium is not reabsorbed in the kidney tubules in the same way as sodium and potassium, *i. e.* by hydrogen ion exchange.

The lack of significant effect on the blood pressure, heart rate and respiration rate of Diamox should further stimulate the use of this drug as a diuretic.

## Summary.

A relationship between the effect of the carbonic anhydrase inhibitor, Diamox, on the urinary excretion of sodium and potassium and the plasma bicarbonate concentration has been shown. When the plasma bicarbonate concentration is low, the inhibitor has no effect. It is suggested that this can explain the lack of effect of Diamox after prolonged treatment, since this leads to a lowering in the plasma bicarbonate concentration.

It is shown that there is no qualitative or quantitative difference in the effect of Diamox on the urinary electrolytes in the normal and the adrenalectomized dog.

The drop in glomerular filtration rate which is occasionally caused by Diamox is presumably caused by a simultaneous drop in blood pressure.

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# On the Behavior of the "Sodium Pump" in Frog Skin at Various Concentrations of Na Ions in the Solution on the Epithelial Side.

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Na ions are known to pass through frog skin from the outside (epithelial side) to the inside (corium side) with greater ease than in the opposite direction, even with fairly dilute solutions on the outside and against the electrochemical potential gradient of Na ions (USSING 1949). This ability of the skin to transport Na ions actively is supposed to be connected with a sodium transport system, or a "sodium pump" linked up with and dependent on the metabolism of the cells.

HUF (1935, 1936) first showed that frog skin bathed with Ringer's solution on both sides transported salt from the outside to the inside, and KROGH (1937) demonstrated that the skin of a living frog was able to take up NaCl from a very dilute solution of less than 1 mM (cf. also USSING 1953). USSING and ZERAHN (1951) showed the way to treat the process quantitatively when they designed a method for determining the "active transport potential" of the sodium pump by means of radioactive isotopes. In a further analysis, LINDERHOLM (1952) developed other methods for the determination of the essential parameters of the sodium pump, as well as a quantitative treatment of the transport of NaCl by the skin from measurements with electrical and radioactive isotope methods. Investigations recently reported by HUF and

WILLS (1953) and by LINDERHOLM (1953) confirm the essential correctness of the latter method as regards salt transport.

The purpose of this paper is to study the effect of variation in the concentration of the outside solution on the sodium pump and on other properties of isolated frog skin. Facts are brought forward which establish some essential features of a "hypothetical frog skin" proposed previously (LINDERHOLM 1952).

## Methods.

The experimental arrangement was in general the same as described by LINDERHOLM (1952). Green frogs (*Rana temporaria*) were used. They were collected during the autumn and kept without food in water tanks in a cold cellar until January, when the experiments were performed. Abdominal skin of the frogs was prepared and mounted in a special chamber. The solutions in the two compartments (1 and 2) of the chamber on either side of the skin were kept circulating by bubbling air through them. The solution on the inside (2) was always a frog Ringer's solution containing a phosphate buffer, pH 7.5, made up from 9 parts of a solution containing 111.15 mM NaCl, 3.60 mM KCl, and 3.81 mM/2 $CaCl_2$, and one part of the 100 mM phosphate buffer made up of $Na_2HPO_4$ and $NaH_2PO_4$ in proportions 16.16/1.92. The solution on the outside (1) was either the same Ringer's solution or this solution diluted with distilled water in various proportions. The volumes of the solutions in 1 and 2, usually about 8 ml, could be measured by means of calibrated funnels. The temperature of the bathing solution was $20°.0 \pm 0°.1$ C.

*Performance of the experiments:* After being mounted in the chamber, the skins were allowed to stabilize for 2 hours. The concentration of the solution in 1, $c_1$, was kept low, usually about 1—2 mM, during this period. The solutions on the two sides were then changed. To compartment 1 of the chamber was added a Ringer's solution diluted to a concentration corresponding to about 0.2 mM of sodium chloride. To compartment 2 was added Ringer's solution of the composition described above, but containing the radioactive isotopes corresponding to relative activity of $2 \times 10^5$ counts/min./ml of $Na^{24}$ and $3 \times 10^4$ counts/min./ml of $Cl^{36}$. (The geometrical efficiency of the counter being about 15 per cent.) After about 15 minutes samples were drawn from 1 (0.4 ml) and from 2 (0.1 ml) by means of semiautomatic Carlsberg pipettes, to determine the radioactivity. After an interval of about 30 minutes samples were again taken for the same purpose, and once more after another 30 minutes. Then a certain amount of Ringer's solution was added to 1 to give a higher concentration, and after 15 minutes the sampling was repeated for two further 30 minutes periods, after which Ringer's solution was again added to 1. In this way the concentration in 1 was increased stepwise, until finally all the solution in 1 was replaced by Ringer's solution. The concentration levels, $c_1$, at which

measurements were made were approximately the following: 0.4, 1.9, 5.8, 18.4, 54.5, and 110 mM (cf. Tables I and II). These values were obtained from conductivity measurements in 1, and expressed as the concentration of a NaCl solution which at the same temperature would have the same conductivity as the solution in question.

*Measurements:* The electrical potential across the skin, $\varphi$, the d. c. conductance of the skin, $G$, and the conductivity of the solution in 1 were measured every 5 to 15th minute. The accuracy of the measurements was the same as given earlier. $\varphi$ was measured to the nearest mV, the $G$ measurements were correct within 1—2 per cent or better, and the conductivity measurements for the solution in 1 within 0.1 per cent at concentrations less than approximately 20 mM. Except the symmetrical square wave for the $G$ measurements no electrical current was passed through the skin from an external source.

The flux of Na and Cl ions outwards through the skin was measured by means of the radioactive isotopes $Na^{24}$ and $Cl^{36}$ added to the solution in 2. Samples were taken from the two sides of the skin at about 30 minutes intervals, and the relative activity of the solutions determined by a Geiger-Müller counter. The $Na^{24}$ activity was obtained as the difference between the initial activity and the $Cl^{36}$ activity obtained 1—2 months later when the $Na^{24}$ had decayed. Correction was made for a small amount of radioactive isotopes of long half-life (mainly $Na^{22}$) contaminating the $Na^{24}$. All values were corrected for the resolving time of the counter and for decay.

Some of the $Cl^{36}$ activity values from solution 1 were corrected according to the following principle. If a curve was drawn with the activity of the different samples plotted against the time, a curve was obtained with jumps corresponding to the addition of Ringer's solution to alter the concentration of the solution in 1, $c_1$. As the volumes were known, the magnitude of the activity jump could be estimated. Between the jumps an approximately linear increase with the time was obtained. For some activity values which deviated from the smoothed curve the graphically interpolated values were used.

The radioactivity of the solution in 1 could be neglected as compared with that of the solution in 2, being of the order of 1 per cent or less of the latter. The ionic flux could therefore be calculated according to the following formula (cf. LINDERHOLM 1952, eqn. 2.2.2 p. 53).

$$-\Phi^i_2 = 10^6 \frac{\left\{ v_1^{t\,(n+1)}\, N_1^{t\,(n+1)} - (v_1^{t\,(n)} - q_1)\, N_1^{t\,(n)} \right\} c^i_2\, q_2}{q_1\, \bar{N}_2\, \Delta t\, A}$$

$\mu\mu$mol cm$^{-2}$ sec$^{-1}$. (eqn. 1)

$-\Phi^i_2$ is the total flux of an ion $B^i$ from 2 to 1. (B symbolizes a chemical component.) $v_1^{t\,(n)}$ and $v_1^{t\,(n+1)}$ are the volumes in solution 1 in ml at the time when the $n$th and $(n+1)$th sample were taken, and $N_1^{t\,(n)}$ and $N^{t\,(n+1)}$ are the relative activities in counts per minute of the ion $B^i$ in the same samples. $\bar{N}_2$ is the relative activity of the isotope in the samples drawn from 2, interpolated to correspond to the mean activity

during the period $\Delta t = t^{(n+1)} - t^{(n)}$ in sec. All activities are corrected for decay. $q_1$ is the volume in ml of the pipette used in 1, and $q_2$ the corresponding volume for the pipette used in 2. $c_2^i$ is the concentration of the ion in question in 2 in mM. $A$ is the area of the skin in cm² (7.1 cm²).

The net flux of salt through the skin, $\Phi^{NaCl}$, was calculated from changes in $c_1$ according to the formula (cf. LINDERHOLM 1952, eqn. 2.2.1, p. 48)

$$\Phi^{NaCl} = 10^6 \frac{\Delta c_1 v_1}{A \Delta t} \mu\mu\text{mol cm}^{-2} \text{ sec}^{-1} \ldots\ldots \text{ eqn. 2}$$

where $\Delta c_1 = c_1^{i\,(m)} - c_1^{i\,(m+1)}$ is the difference between the concentration in mM of the solution in 1 at the time of the $m$th and $(m+1)$th measurement and $\Delta t = t^{(m+1)} - t^{(m)}$, the corresponding time interval in seconds. $v_1$ is the volume in 1 during $\Delta t$. ($\Phi^{NaCl}$ was also estimated by another method, see below.)

## Theory.

The experimental data are treated in the same way as previously (LINDERHOLM 1952). The frog skin is regarded as an uncharged or non-ionic (in the sense of TEORELL 1951, 1953), homogeneous membrane with well stirred solutions on the two sides. Cl ions and some of the Na ions diffuse passively through the skin, perhaps through pores of ionic dimensions (cf. GARBY and LINDERHOLM 1953). The skin is further supposed to contain a "sodium pump". It consists of a carrier system to which Na ions are specifically bound and in which Na ions may be transported in both directions. The pump also includes a force which tends to transport Na ions in the carrier system inwards through the skin. The effect of this force is expressed as an active transport potential.

Though the Ringer's solution used contains other ions than Na and Cl, earlier experiments seem to show that they are of little importance quantitatively and that the electrical d. c. conductance through the skin is essentially due to Na and Cl ions (LINDERHOLM 1952). The other ions are therefore neglected.

These assumptions, which will be discussed later, lead to the equations derived in an earlier paper (LINDERHOLM 1952). The equations by which the different parameters of the skin were calculated in the present experiments are summarized below. (In brackets after the number of the equation is the number of the corresponding equation in the earlier paper.)

The partial conductance of Cl ions through the skin, $G^{Cl}$, was calculated from

$$G^{Cl} = \Phi_2^{Cl} \frac{F\left(\frac{a_1\xi}{a_2} - 1\right)}{\frac{RT}{F} \ln \frac{a_2}{a_1\xi}} \quad \text{eqn. 3 (1.3.14)}$$

$a_1$ and $a_2$ are the activities of the ion in question in the solutions in 1 and 2 respectively. $R$ is the gas constant, $T$ the absolute temperature, and $F$ the Faraday. $\xi$ is defined by

$$\varphi = \varphi_2 - \varphi_1 = (RT/F) \ln \xi \quad \text{eqn. 4 (1.2.2)}$$

where $\varphi_1$ and $\varphi_2$ are the electrical potentials in solution 1 and 2 respectively.

The partial conductance of Na ions, $G_a^{Na}$, was obtained from

$$G_{a\,I}^{Na} = G - G^{Cl} \quad \text{eqn. 5 (1.3.4)}$$

$a$ as a subscript is used in connection with symbols referring to the actively transported component.

The active transport potential for Na ions through the skin $\varphi_a^{Na}$ was obtained from

$$\varphi_{a\,I}^{Na} = \frac{\varphi\,(G_a^{Na} + G^{Cl}) + (G_a^{Na} - G^{Cl})\,(RT/F)\,\ln\,(a_2/a_1)}{G_a^{Na}} \quad \text{eqn. 6 (1.5.4)}$$

or from

$$\varphi_{a\,II}^{Na} = \varphi + \frac{RT}{F} \ln \frac{a_2}{a_1} + \frac{RT}{F} \ln \left\{ 1 - \frac{\Phi_2^{Cl}}{\Phi_{a_2}^{Na}} \left( 1 - \frac{a_1\xi}{a_2} \right) \right\} \quad \text{eqn. 7 (1.7.2)}$$

Besides the calculation from eqn. 5, the partial conductance of Na ions was also calculated from

$$G_{a\,II}^{Na} = \Phi_{a\,2}^{Na} \frac{F\left(\frac{a_1 \xi_a^{Na}}{a_2\,\xi} - 1\right)}{\frac{RT}{F} \ln \frac{a_2\,\xi}{a_1\,\xi_a^{Na}}} \quad \text{eqn. 8 (1.4.3)}$$

where $\xi_a^{Na}$ is obtained from Na

$$\varphi_{a\,II}^{Na} = (RT/F) \ln \xi_a^{Na} \quad \text{eqn. 9 (1.2.8)}$$

Besides eqn. 2, the following eqn. was used for $\Phi^{NaCl}$:

$$\Phi^{NaCl} = \Phi_a^{Na} = \Phi^{Cl} = \frac{G_a^{Na} G^{Cl} \left\{ \varphi_a^{Na} - 2\,(RT/F)\,\ln\,(a_2/a_1) \right\}}{F\,G} \quad \text{eqn. 10 (1.5.5)}$$

where $\Phi^{NaCl}$, $\Phi_a^{Na}$ and $\Phi^{Cl}$ are the net fluxes of the respective components.

The two different ways in which some of the quantities may be evaluated provides some check on the validity of the equations in the present case and justifies using them with some degree of confidence.

If the fraction of Na ions which diffuses passively and that which is actively transported through the skin were known, it would be possible to estimate the "true transport potential of the sodium pump". These fractions cannot be distinguished experimentally, but some more or less probable assumptions can be made about them (cf. below). The partial conductance carried by passively diffusing Na ions, $G_p^{Na}$, is assumed to be equal to $G^{Cl} \times (u^{Na}/u^{Cl})$, where the mobility ratio of the two ions is assumed to be the same as in water (= 0.66). The assumptions leads to

$$\varphi_a^{Na} = \varphi_A^{Na}\, G_A^{Na}/(G_A^{Na} + G_p^{Na}), \quad \ldots\ldots\ldots \text{ eqn. 11 (1.6.3)}$$

where $G_A^{Na} = G_a^{Na} - G_p^{Na}$ represents the partial conductance of Na ions through the sodium pump. $A$ as a subscript is used in connection with symbols referring to ions which pass through the sodium pump while $p$ refers to passively diffusing Na ions.[1]

A diagrammatic representation of some of the ideas included in the equations is given in Fig. 1.

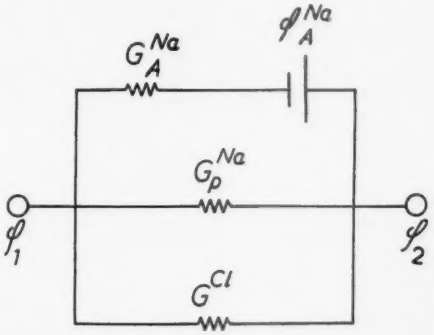


Fig. 1. An equivalent scheme showing the different ways in which ions may pass through the skin. The total potentials (in electrical terms) corresponding to the forces which move the ions (from 1 to 2 for positive values) are as follows:
for Cl ions: $(RT/F)\ \ln\ (a_1/a_2) + \varphi$
for passive Na ions: $(RT/F)\ \ln\ (a_1/a_2) - \varphi$
for Na ions passing through the sodium pump: $(RT/F)\ \ln\ (a_1/a_2) - \varphi + \varphi_a^{Na}$
The resulting net ionic flux $(F \times \ldots)$ is the product of the partial conductance, $G_A^{Na}$, $G_p^{Na}$ or $G^{Cl}$, and the respective potential term.

[1] $A$ is used as a subscript here instead of $a$ in the earlier paper (LINDERHOLM 1952).

Table

| $t$ min | $\mathcal{V}$ mV | $c_1$ mM | $-\phi^{Na}_{a_2}$ | $-\phi^{Cl}_{2}$ | $G$ |
|---|---|---|---|---|---|
| 0— 21..... | + 12 | 0.40 | | | 0.113 |
| 21— 51..... | + 15 | 0.48 | 20.5 | 10.7 | 0.118 |
| 51— 81..... | + 18 | 0.63 | 20.8 | 11.1 | 0.120 |
| 96—125..... | + 37 | 1.87 | 23.8 | 7.9 | 0.151 |
| 125—155..... | + 34 | 1.88 | 34.3 | 4.8 | 0.142 |
| 172—200..... | + 49 | 5.84 | 33.1 | 12.9 | 0.173 |
| 200—231..... | + 49 | 5.86 | 32.3 | 11.2 | 0.176 |
| 245—275..... | + 70 | 18.4 | 39.6 | 3.2 | 0.214 |
| 275—304..... | + 70 | 18.4 | 33.8 | 4.9 | 0.222 |
| 321—349..... | + 82 | 54.7 | 41.3 | 3.2 | 0.338 |
| 349—382..... | + 83 | 54.7 | 89.2 | 3.6 | 0.353 |
| 399—426..... | + 76 | 110 | 75.4 | 4.8 | 0.477 |
| 426—458..... | + 71 | 110 | 69.0 | 5.4 | 0.470 |

Table

| $t$ min | $\mathcal{V}$ mV | $c_1$ mM | $-\phi^{Na}_{a_2}$ | $-\phi^{Cl}_{2}$ | $G$ |
|---|---|---|---|---|---|
| 0— 21..... | 0 | 0.35 | | | 0.072 |
| 21— 50..... | 0 | 0.44 | 24.4 | 19.9 | 0.073 |
| 50— 86..... | + 7 | 0.53 | 27.0 | 20.5 | 0.079 |
| 98—127..... | + 30 | 1.88 | 30.5 | 17.6 | 0.133 |
| 127—152..... | + 33 | 1.92 | 49.6 | 17.6 | 0.140 |
| 170—200..... | + 47 | 5.87 | 42.9 | 13.9 | 0.191 |
| 200—235..... | + 47 | 5.89 | 30.2 | 14.0 | 0.202 |
| 256—278..... | + 60 | 18.4 | 66.9 | 8.6 | 0.269 |
| 278—305..... | + 63 | 18.4 | 38.1 | 8.3 | 0.295 |
| 320—449..... | + 62 | 54.5 | 50.4 | 9.5 | 0.375 |
| 349—379..... | + 57 | 54.5 | 49.0 | 8.9 | 0.380 |
| 391—419..... | + 43 | 110 | 55.5 | 18.5 | 0.445 |
| 419—449..... | + 38 | 110 | 65.5 | 19.6 | 0.451 |

The tables represent the same experiments as those in Figs. 2 and 3. At zero time ($t = 0$) the radioactive isotopes were added to the solution in 2. Unfortunately, the concentration of the solution in 1, $c_1$, was changed at the same time from about 1—2 mM, at which $c_1$ the skin had stabilized for 2 hours, to about 0.4 mM. This probably resulted in erroneous values for $\phi_{NaCl}$ as calculated from eqn. 2 during the first periods (see text). The values are mean values for the respective time intervals. The $G$ values are given in $k\Omega^{-1}$, $cm^{-2}$, and the $\phi$ values in $\mu\mu mol\ cm^{-2}\ sec^{-1}$.

I.

| $G^{Cl}$ (eqn. 3) | $G^{Na}_{a\,I}$ (eqn. 5) | $G^{Na}_{a\,II}$ (eqn. 8) | $\gamma^{Na}_{a\,I}$ mV (eqn. 6) | $\gamma^{Na}_{a\,II}$ mV (eqn. 7) | $\varphi_{NaCl}$ (eqn. 10) | $\varphi_{NaCl}$ (eqn. 2) |
|---|---|---|---|---|---|---|
| | | | | | | — 345 |
| 0.0087 | 0.1093 | 0.056 | 138 | 129 | — 10.6 | — 132 |
| 0.0098 | 0.1102 | 0.056 | 134 | 125 | — 11.0 | — 52 |
| 0.0113 | 0.1397 | 0.075 | 131 | 127 | — 7.2 | — 17.2 |
| 0.0067 | 0.1353 | 0.123 | 130 | 129 | — 4.5 | — 19.0 |
| 0.0329 | 0.1401 | 0.108 | 115 | 114 | — 7.6 | — 13 |
| 0.0285 | 0.1475 | 0.108 | 116 | 114 | — 6.5 | — 12 |
| 0.0222 | 0.1918 | 0.159 | 117 | 117 | + 6.1 | 0[1] |
| 0.0337 | 0.1883 | 0.144 | 118 | 120 | + 9.3 | 0[1] |
| 0.0568 | 0.2812 | 0.225 | 113 | 116 | + 37.9 | |
| 0.0649 | 0.2881 | 0.418 | 115 | 111 | + 43.9 | |
| 0.117 | 0.360 | 0.439 | 101 | 96 | + 92.1 | |
| 0.115 | 0.355 | 0.407 | 94 | 91 | + 84.5 | |

II.

| $G^{Cl}$ (eqn. 3) | $G^{Na}_{a\,I}$ (eqn. 5) | $G^{Na}_{a\,II}$ (eqn. 8) | $\gamma^{Na}_{a\,I}$ mV (eqn. 6) | $\gamma^{Na}_{a\,II}$ mV (eqn. 7) | $\varphi_{NaCl}$ (eqn. 10) | $\varphi_{NaCl}$ (eqn. 2) |
|---|---|---|---|---|---|---|
| | | | | | | — 144 |
| 0.0141 | 0.0589 | 0.045 | 102 | 93 | — 19.7 | — 71 |
| 0.0159 | 0.0831 | 0.056 | 106 | 102 | — 20.2 | — 50 |
| 0.0231 | 0.1099 | 0.061 | 114 | 109 | — 16.4 | — 17.1 |
| 0.0241 | 0.1159 | 0.148 | 118 | 118 | — 16.3 | — 28.8 |
| 0.0342 | 0.1568 | 0.146 | 113 | 113 | — 8.5 | — 12 |
| 0.0347 | 0.1673 | 0.097 | 113 | 110 | — 8.6 | — 19 |
| 0.0467 | 0.2223 | 0.267 | 107 | 106 | + 8.0 | 0[1] |
| 0.0453 | 0.2497 | 0.130 | 110 | 101 | + 9.1 | 0[1] |
| 0.0994 | 0.2756 | 0.270 | 96 | 96 | + 45.5 | |
| 0.0808 | 0.2992 | 0.246 | 85 | 88 | + 32.8 | |
| 0.187 | 0.258 | 0.348 | 74 | 66 | + 82.9 | |
| 0.176 | 0.275 | 0.367 | 62 | 56 | + 69.1 | |

The different values of parameters which could be estimated in two more or less independent ways are given for comparison. The number of the equations from which they were obtained are given within brackets.

The $c_1$ values are those obtained from conductivity measurements, and the Cl concentration is therefore slightly lower and the Na concentration slightly higher than the figures given.

[1] These values are uncertain but should not deviate more than about $\pm 10$ from zero.

## Results.

Independently of assumptions and theoretically derived equations, some results indicate a facilitated movement or mobility of Na ions as compared with Cl ions in both directions through the skin at a low $c_1$. Outward and inward total fluxes of Na and Cl ions have not been measured at the same time in one skin, but if similar skins are used and the flux is measured inwards in one skin and outwards in another, some information is obtained. The outward flux values of the experiments in Table I and II at $c_1$ about 5.9 mM may thus be compared with the inward flux values from earlier similar experiments performed with $c_1$ at 7—7.5 mM, $\varphi = 54$ mV, and $G = 0.25$ k$\Omega^{-1}$cm$^{-2}$ (mean of 4 experiments, Table 8 and 9, LINDERHOLM 1952). The inward Na flux was 41 and the corresponding Cl flux 15 $\mu\mu$mol cm$^{-2}$ sec.$^{-1}$. These values are of the same magnitude as the outward fluxes (see the Tables) and agree well with the low $\Phi^{NaCl}$ in these experiments. The Na and Cl ions should thus be near flux equilibrium (*i. e.* net flux about zero), and the partial conductances of the ions should then be nearly proportional to the total fluxes (cf. HODGKIN, as cited by KEYNES 1949, and LINDERHOLM 1952, eqn. 1.3.15). The Na fluxes are at least twice as large as the Cl fluxes and the same ratio should thus be valid for the partial conductances.

Though the experiments are not quite comparable, the $c_1$, $G$, and $\varphi$ values, for instance, being slightly different, the results are in rough conformity with the assumptions included in the theoretical part. More detailed information about the skin and its different parameters at various $c_1$ values is obtained from calculations according to the equations in the theoretical part.

Such results from two experiments are given in Figs. 2 and 3 and in Tables I and II. These two experiments are the only ones performed, but they agree well with each other and also with earlier experiments in the $c_1$ ranges investigated. The results indicate that the treatment outlined in the theoretical part is possible to apply and approximately correct. As in an earlier paper (LINDERHOLM 1952) one of the best arguments seems to be that the sum of the partial conductances of Na and Cl ions ($G_{a\,II}^{Na} + G^{Cl}$), calculated on the basis of radioactive flux measurements agrees satisfactorily with the electrically measured

d. c. conductance $G$ (Tables I and II) in the whole $c_1$ range and for the wide variation of electrical and chemical potential differences across the skin. Another argument is that this treatment makes it possible to reconstruct the behaviour of the skin quite well with a few simple assumptions (see below in connection with the hypothetical skin).

The general form of the $\varphi/\log(c_1)$ and $G/\log(c_1)$ curves is the same as found in many earlier experiments made by the author, cf. also HASHIDA (1922), GREVEN (1942), and LINDERHOLM (1952). With regard to the long duration of the experiments — about 6 hours — it is remarkable how well the curves agree with those obtained from experiments in which the sequence of various (increasing or decreasing) concentrations of the solution in 1 was much more rapid. This seems to indicate that the properties of the skins did not change much during the long experiment. The $G$ values of the skins are low, but within the rather wide limits of normal variation.

For decreasing $c_1$, $G$ — in these as in earlier experiments — is found usually to decrease less than would be expected if the skin were a homogeneous uncharged membrane. For such a membrane, $G_r$ should be approximately constant for varying $c_1$ (see below).

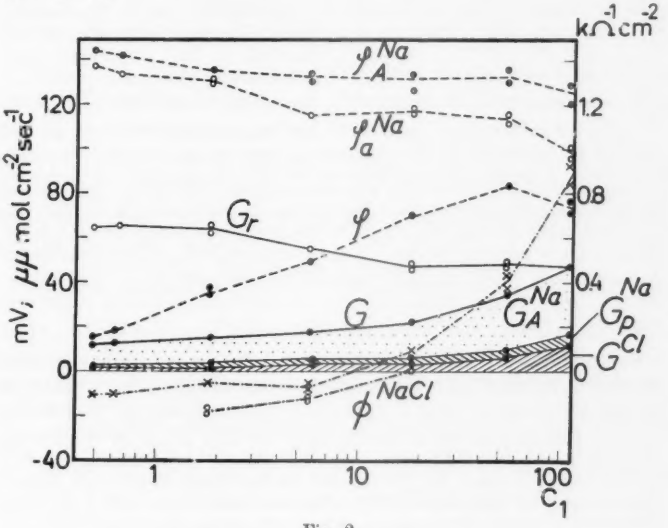


Fig. 2.

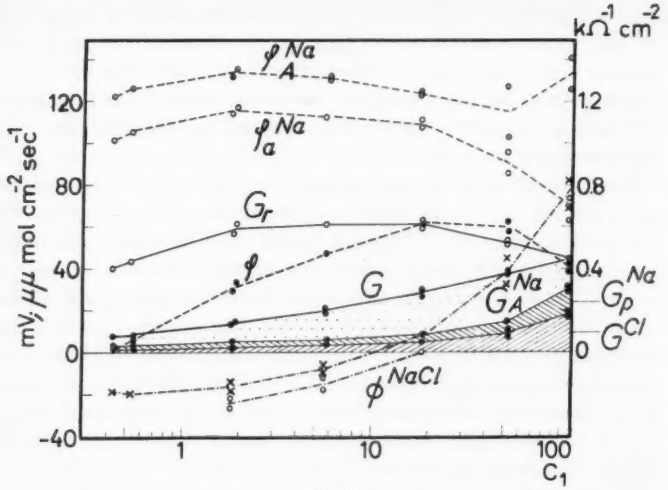


Fig. 3.

Figs. 2 and 3. The figures represent experiments on two skins. The abscissae are the concentrations of the solution on the outside of the skin, $c_1$, in mM on a logarithmic scale. Each dot represents the mean value over a period of about half an hour. The total d. c. conductance of the skin, $G$, was measured electrically. The form of the $G$ curve in Fig. 2 is that which agrees best with most of the earlier experiments. The height of the lowest shaded area represents the partial conductance of Cl ions $G^{Cl}$, and is calculated from the $Cl^{36}$ flux values. The height of the shaded area above represents the partial conductance of passive Na ions, $G_p^{Na} = (u^{Na}/u^{Cl}) \times G^{Cl} = 0.66 \times G^{Cl}$, according to the assumptions given in the text. The height of the dotted area represents the partial conductance of Na ions through the sodium pump, $G_A^{Na}$, (cf. eqn. 11), and the sum $G_A^{Na} + G_p^{Na} = G_a^{Na}$ is the partial conductance of Na ions through the skin (cf. eqn. 5). $G_r$ is obtained from $G$ multiplied by a function of $c_1$ (see text), and should be a straight line parallel to the $c_1$-axis for a membrane which is homogeneous, uncharged, and without carrier systems. $\phi_{NaCl}$ is represented by two curves. The values for the shorter one are obtained from eqn. 2, while the complete curve is obtained from eqn. 10. $\varphi$ is the directly measured electrical potential difference across the skin. $\varphi_a^{Na}$, the active transport potential of the skin for Na ions, is obtained from eqn. 6, and $\varphi_A^{Na}$, the "true transport potential of the sodium pump", from eqn. 11.

It may be asked how the conductance of a homogeneous uncharged membrane varies under conditions corresponding to the present experiments with frog skin, *i. e.* when one side of the membrane is in contact with a solution of a strong 1-1-electrolyte of constant concentration and the other side with a series of dilutions of the same solution. Expressions for membrane conductance have been derived theoretically by GOLDMAN (1943), HODGKIN and KATZ (1949), TEORELL (1951) and others. When there is a great difference in concentration

of the solutions on either side of a membrane, corrections for activity coefficients are probably of importance, though our incomplete knowledge of the activities inside the membrane make such corrections of doubtful value.

Nevertheless, such a correction for conditions corresponding to the experiments was tried by LINDERHOLM (1952, eqn. 1.3.12). The conductance carried by a single 1-1-electrolyte or the partial conductance carried by one of the ions through the membrane is then expressed by

$$G^{i} = \frac{aF^2}{\delta} \cdot \frac{u^{i}\{(c_2 - c_1) - \psi\}}{\ln (a_2/a_1)} \quad \underset{(c_1 = c_2)}{=} \quad \frac{aF^2}{\delta} \cdot u^{i}c. \qquad \text{..eqn. 12}$$

where

$$\psi = 1.15\left[\left(\sqrt{c_2} - \frac{1}{1+\sqrt{c_2}}\right) - \left(\sqrt{c_1} - \frac{1}{1+\sqrt{c_1}}\right) + 2 \ln \frac{1+\sqrt{c_1}}{1+\sqrt{c_2}}\right],$$

$a$ being the "effective diffusion area", $\delta$ the thickness of the membrane and the other symbols have the same meaning as before.

Eqn. 12 is used here to describe the variation in conductance for varying concentration on one side of a homogeneous, uncharged membrane. The $G_r$ values are obtained by multiplying the $G$ values by the function $\{c_2 \ln (a_2/a_1)\} : \{(c_2 - c_1) - \psi\}$ (cf. LINDERHOLM 1952, p. 80).

Which of the theoretically derived expressions for membrane conductance for a homogeneous uncharged membrane corresponds best to reality awaits experimental decision, but it should be noted that the relative change in membrane conductance under conditions like those of the present experiments is the same, within 5—10 per cent, whether the correction for activities is applied or not.

If the $G^{Cl}$ values are examined according to the same principles as the $G$ values above, it is found that they decrease more rapidly for decreasing $c_1$ than would be expected if the skin behaved as a passive, homogeneous and uncharged membrane for Cl ions. The $G_a^{Na}$ values, on the other hand, decrease much less than would be expected for such a membrane. These results will be discussed later.

The $\Phi^{NaCl}$ values calculated according to eqn. 2 (from conductivity measurements in 1) are obtained only in a limited range. At the lowest concentrations ($c_1 \approx 0.5$ mM), quite high negative $\Phi^{NaCl}$ values were obtained for the first period after addition of the dilute solution. During the following periods $\Phi^{NaCl}$ from eqn. 2 rapidly approached the values obtained by eqn. 10 (cf. Tables I and II). The results at the lowest $c_1$ are probably erroneous, and this is evidently due to a too short period of equilibration at this low concentration and presumably to leakage of electrolytes out of the skin. These errors probably influence

the flux of the radioactive isotopes much less. At $c_1$ above 20 mM the conductance measurements were too inacurate to allow any conclusions.

The $\Phi^{NaCl}$ values calculated according to eqn. 10 (from radioactive flux measurements) cover the whole $c_1$ range. They are in general slightly higher than those obtained by eqn. 2. This might be expected, however, as several sources of error, such as leakage of ions out of the skin, a small net flux of water from 1 to 2, and finally, though probably negligible, a leakage of ions from the KCl bridge for potential measurements tend to make the values calculated from eqn. 2 too low. The good agreement between the results obtained quite independently is thus quite satisfying, and is a good check on the applicability and approximate correctness of equations used.

In the skins investigated $\Phi^{NaCl}$ is zero for $c_1$ about 10 mM. For higher $c_1$ there is a net transport of salt inwards through the skin, while at lower $c_1$ the skin leaks salt outwards.

$\varphi_a^{Na}$ was calculated in two ways, from eqn. 6 and eqn. 7. The two sets of values agree fairly well (see the Tables). $\varphi_a^{Na}$ decreases when $c_1$ approaches $c_2$. This might be expected if a reasoning like that which leads to eqn. 11 is valid, and the "true active transport potential" of the sodium pump, $\varphi_A^{Na}$ is constant and independent of $c_1$. On the other hand, if $\varphi_A^{Na}$ is calculated according to the assumptions given in connection with eqn. 11 it is found to be approximately constant in the two experiments. These $\varphi_A^{Na}$ values are within the range of the corresponding values obtained earlier (LINDERHOLM 1952), though slightly low.

$G_A^{Na}$ decreases with decreasing $c_1$, but not as much as $G_a^{Na}$, and not at all as much as would be expected for the conductance of an electrolyte through an uncharged homogeneous membrane under similar conditions.

## Discussion.

The present experiments describe the behaviour of isolated frog skin for varying $c_1$. As the results indicate, it was possible to apply the treatment outlined in the theoretical part for a wider $c_1$ range than has been tried previously. Some essential features of the "hypothetical skin" put forward in an earlier paper (LINDERHOLM 1952, p. 78) were established. Divergences from theory will be discussed.

To treat an inhomogeneous biological membrane like the frog skin with its different layers of epithelium, connective and glandular tissue as a membrane that is homogeneous as regards diffusion processes, is certainly open to criticism. However, there are some facts which make the treatment more reasonable.

Electrical impedance measurements on frog skin seem to indicate that there is one structure that is essentially responsible for its impedance. The impedance locus is almost a semicircle, and correspondingly the transient of a current or voltage step impact is approximately exponential (TEORELL 1946, 1949, and unpublished experiments by the author). The impedance values almost require a single structure of high capacity and high resistance which constitutes the essential diffusion resistance. The high capacity of frog skin makes it probable that this structure is a very thin membrane. It is difficult to imagine two or more structures with so similar time constants as the impedance values imply. Deviations from the ideal behaviour described were found earlier, and led to the postulation of several layers or different kinds of capacities (cf. GILDEMEISTER 1919, LULLIES 1928 and others). They may partially depend on the experimental methods used, *e. g.* effects at the border of the electrodes. They are also more pronounced in limited frequency ranges.

A recent investigation gives further evidence for the single structure view. OTTOSON, SJÖSTRAND, STENSTRÖM and SVAETICHIN (1953) were able to detect in electronmicroscopic pictures a special submicroscopic basement membrane 200—300 Å thick which covers, as a continuous membrane, the basal epidermal cells, and demarcates the corium connective tissue from the epithelium. It is separated from the epidermal cells by a space of about the same width as the thickness of the membrane, and is of the same type as a previously described membrane in the kidney tubule of mouse (SJÖSTRAND and RHODIN 1953). The epithelial cells are separated by intercellular spaces and the individual cells are delimited by a continuous cell membrane. They further showed by combining elaborate optical and microelectrode techniques that the essential electrical potential drop is located at the dermo-epidermal junction. It therefore seems rather tempting to assume that the basement membrane is the site of the active transport as well as of the essential diffusion resistance.

USSING (1953) states that the epithelium of the skin contains much potassium. Then it seems unlikely that the active sodium transport takes place through these cells. Perhaps ions that pass through the skin diffuse mainly between the epidermal cells and the basement membrane situated outside the cells, though probably in close connection with them, may be the site of the sodium pump. The reactions at the surface of the membrane which must be assumed for the active transport mechanism may still be driven by the cells in the vicinity of the basement membrane.

As already mentioned, $G^{Cl}$ decreases more rapidly with decreasing $c_1$ than would be expected if Cl ions diffused through a homogeneous uncharged membrane. This should not be stressed too much, however, as the living skin may have changed during the 6 hours the experiments lasted. It is not unusual that the conductance of the skin increases with time. Experiments should have been made beginning with high $c_1$ for comparison.

$G_a^{Na}$ decreases much less with decreasing $c_1$ than would be expected for a homogeneous uncharged membrane. At a first glance, the behaviour of $G^{Cl}$ and $G_a^{Na}$ would correspond qualitatively to a negatively charged membrane. GREVEN (1942) postulates a membrane charge of about 6 mEqv./l or more in order to explain the form of the $\varphi/\log(c_1)$ curve by the Teorell-Meyer-Sievers theory. With such a relatively high charge, one would expect a more rapid decrease of $G^{Cl}$ for the lowest $c_1$ in the experiments. There are also other reasons to regard the frog skin not as a charged membrane in the sense of Teorell-Meyer-Sievers but instead as an essentially uncharged membrane containing a carrier with a specific affinity only for Na (and Li) ions (cf. LINDERHOLM 1952, p. 76—82).

As already mentioned, the $\varphi/\log(c_1)$ curve has the same general form in these experiments as in earlier ones of the same type. One observation as to the $c_1$ value for which $\varphi$ is found to be a maximum, may be worth mentioning. With high conductance skins the maximum $\varphi$ is often at a lower $c_1$ than with skins of low conductance, cf., for instance, two examples given by LINDERHOLM (1952, p. 81). A similar result was obtained in some calculations on a "hypothetical skin", see below, when the total conductance was varied.

Three parameters, $G_A^{Na}$, $G_p^{Na}$, and $\varphi_A^{Na}$ cannot be determined experimentally. They are obtained on an assumption

which is uncertain and questionable. But it leads to results which seem plausible and in a way sustain the assumption. As previously stated, the assumption is that $G_p^{Na} = 0.63 \times G^{Cl}$, where 0.66 is the ratio of the mobilities $u^{Na}/u^{Cl}$ in water. It gives a fairly constant value for $\varphi_A^{Na}$ over the whole $c_1$ range. A constant $\varphi_A^{Na}$ seems reasonable, as it is probable that the metabolic processes — perhaps a potential difference between two redox systems — which may be assumed to drive the sodium pump, would be essentially independent of $c_1$.

Another argument for the assumption is that a membrane which allows Cl ions to pass passively is likely to let Na ions through as well. The ratio between the mobilities of the two ions in the skin may be quite different from that of the mobilities in water, which, however, may be used as a first approximation. With regard to the nearly constant $\varphi_A^{Na}$, it may be approximately correct.

Finally, if the sodium pump is stopped, it may be expected that $G_A^{Na}$ approaches zero (cf. below in connection with considerations about the mechanism of the sodium pump). This is actually the case in several experiments in which the active transport was inhibited (reversibly) by mersalyl (except in "short-circuited" skins) and $CO_2$ (LINDERHOLM 1952) and by dinitrophenol (LINDERHOLM, unpublished experiments).

Beside the passive Na ions, assumed to pass through the skin along the same paths as Cl ions, Na ions are supposed to be transported actively through the skin, combined with a carrier which specifically absorbs Na ions. Some features of this process will now be discussed.

### On the Nature of the Sodium Pump.

LINDERHOLM (1952) stated that the concept of a redox pump of the type described by CONWAY (1951, 1953) performing the active transport of Na ions was in good agreement with experimental results. Such a pump may be characterized by the resistance it offers to the Na ions passing through it and the force with which it acts on the Na ions. A galvanic cell with its internal resistance (conductance) and electromotive force is taken as a model.

An attempt will be made here to analyze the conditions under which such a pump would give an internal conductance as a

function of the concentration of Na ions on one side of the type estimated from the experiments. A cyclic reaction of a type similar to that working in the redox pump is supposed to take place in the skin. However, there may also be other chemical reactions at work. A paper by SOLOMON (1952) on ion transport through erythrocyte membranes has been borne in mind when the following equations were worked out. The transport mechanism with the assumptions included is described as follows.

At surface 1 of the membrane where active transport takes place, a carrier $X^-$ with specific affinity for Na ions is supposed to be formed according to

$$P + B^1 \underset{k_{-0}}{\overset{k_0}{\rightleftharpoons}} X_1^- + B^2 \quad \text{..... eqn. 13}$$

where $B^1$ and $B^2$ are metabolites or enzymes and P is a precursor of the carrier. Na ions combine with $X^-$ according to

$$Na_1^+ + X_1^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} NaX \quad \text{..... eqn. 14}$$

where $Na_1^+$ signifies Na ions at surface 1. The sodium carrier complex, NaX, diffuses to surface 2 of the active membrane in an essentially unionized form. It is supposed that this diffusion process requires a low activation energy as compared with the reactions taking place at the two surfaces, *i. e.* there is a low diffusion resistance for NaX through the membrane. Consequently NaX has approximately the same concentration at the two surfaces 1 and 2. At surface 2 the following reactions take place.

$$NaX \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Na_2^+ + X_2^- \quad \text{..... eqn 15}$$

where $Na_2^+$ signifies Na ions at surface 2. Further,

$$X_2^- + B_3 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} P + B^4 \quad \text{..... eqn. 16}$$

where $B^3$ and $B^4$ are other metabolites or enzymes. $B^3$ is spatially bound to surface 2 in the same way as $B^1$ is bound to surface 1. $B^1$ has the ability to transform P to $X^-$ and $B^3$ to transform $X^-$

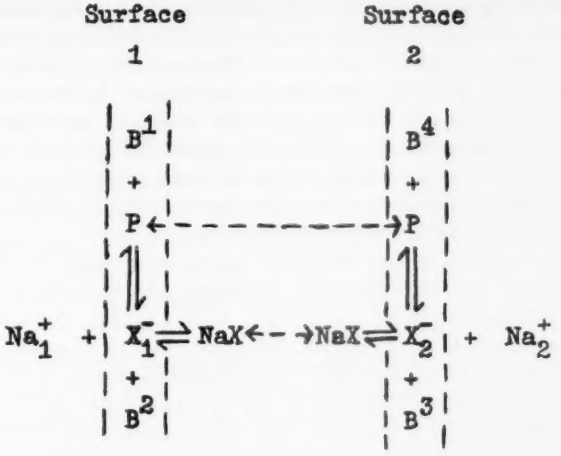


Fig. 4.

to its precursor P, which can diffuse between the two surfaces like NaX. P, $X^-$ and NaX cannot leave the membrane. The cyclic reaction may be driven by coupling to energy delivering metabolic processes via the B-components. Electrons are supposed to be transferred by the reactions 13 and 16. A scheme of the reactions involved is given in Fig. 4. To be identical with the Conway redox pump, $B^3$ should react directly with NaX, and such a model would give similar results in a treatment similar to that which follows.

Applying the law of mass action and assuming the reactions to be monomolecular, the following simultaneous equations valid for the steady state are obtained.

$$\frac{d[X_1^-]}{dt} = k_0[P][B^1] - k_{-0}[X_1^-][B^2] - k_1[Na_1^+][X_1^-] + k_{-1}[NaX] = 0, \quad \text{eqn. 17}$$

$$\frac{d[NaX]}{dt} = k_1[Na_1^+][X_1^-] - k_{-1}[NaX] - k_2[NaX] + k_{-2}[Na_2^+][X_2^-] = 0, \quad \text{eqn. 18}$$

$$\frac{d[X_2^-]}{dt} = k_2[NaX] - k_{-2}[Na_2^+][X_2^-] - k_3[X_2^-][B^3] + k_{-3}[P][B^4] = 0, \quad \text{eqn. 19}$$

To one of the components — for instance, to $Na_2^+$ or a component earlier in the chain of reactions — may be added a $\xi$-factor as an "electrical activity coefficient" (TEORELL 1951, LANGE 1952), to symbolize that the reactions are influenced by the electrical potential difference between the two solutions on either side of the membrane. Such a $\xi$, however, makes the further treatment of eqns 17, 18 and 19 more complicated and is therefore omitted, which may be taken to mean that the variation of $\xi$ with $[Na_1^+]$ is supposed to have a negligible effect on [NaX].

Equations 17, 18 and 19 may be solved with respect to [NaX], which may be expressed as a function of the variable $[Na_1^+]$. The rate constants $k_0$, $k_{-0}$, $k_1$, $k_{-1}$, $k_2$, $k_{-2}$, $k_3$, $k_{-3}$, the activities of the metabolites or enzymes $[B^1]$, $[B^2]$, $[B^3]$, $[B^4]$, and of $[Na_2^+]$ are assumed to be constant and independent of $[Na_1^+]$. It is also assumed that the amount of carrier and its precursor is constant, *i. e.* that $P + X^- + NaX = \text{const.}$ (alternatively that P is constant and independent of $[Na_1^+]$). In order to function well as a carrier, the complex NaX should be little dissociated, *i. e.* the rate constants $k_{-1}$ and $k_2$ should be small, so that $X^-$ may be neglected in this connection. We then arrive at the following expression.

$$[NaX] \times \text{const.} = \frac{b[Na_1^+] + d}{h[Na_1^+] + 1}; \quad \ldots\ldots \text{ eqn. } 20$$

where b, d, and h are constants.

When an electric current is carried through the system from 1 to 2, Na ions at surface 1 combine with $X^-$, which is formed by reaction 13. At surface 2 Na ions are delivered to the solution and $X^-$ is transformed to P by the reaction 16. If current flows in the opposite direction the reverse reactions take place. If a change in the electrical potential between the two solutions is introduced this may be imagined to influence the reactions in one direction by way of a $\xi$-factor, as mentioned above.

The conductance of the carrier system, $G_A^{Na}$, is now proportional to the rate of change in the net flux of Na ions with respect to change in electrical potential difference. If reactions 14 and 15 proceed at equilibrium, *i. e.* when there is no net flux of Na ions through the system, the absolute rate with which Na ions are transformed to NaX or the reverse at the two surfaces is proportionate to $k_{-1}[NaX]$ or $k_2[NaX]$. By analogy to a membrane with an electrolyte in equilibrium on either side, the conductance of which is proportional to the total flux of the ions in

either direction (cf. above under results), the conductance of the carrier system under the conditions mentioned should thus be proportional to [NaX]. Reactions 14 and 15 are supposed to be rate-determining ($k_{-1}$ and $k_2$ have been assumed to be small), and may work far from equilibrium when there is a net flux of Na ions through the system but the conductance $G_A^{Na}$ may still be nearly (dependent on the degree to which the reactions 14 and 15 are rate-determining) proportional to [NaX]. Substituting from eqn. 20 and assuming $c_1 \approx [Na_1^+]$, we obtain

$$G_A^{Na} \approx \text{const} \times [NaX] \approx \frac{bc_1 + d}{hc_1 + 1} \quad \ldots\ldots \text{ eqn. 21}$$

Choosing proper values for the constants in eqn. 21, namely $b = 0.012$, $d = 0.08$, $h = 0.0414$, an expression is obtained which for various $c_1$ gives values for $G_A^{Na}$ in $k\Omega^{-1}$ cm$^{-2}$ which corresponds well to the $G_A^{Na}$ values estimated in earlier experiments (LINDERHOLM 1952) — in which $G_A^{Na}$ was 0.25 $k\Omega^{-1}$ cm$^{-2}$ when $c_1 = c_2$ and 0.13 $k\Omega^{-1}$ cm$^{-2}$ when $c_1$ was 7—7.5 mM (mean of 13 and 4 experiments respectively) — as well as in the present ones. $G_A^{Na}$ obtained in this way is represented graphically by the height of the dotted area in Figs. 5 and 6.

It is not possible to determine the individual rate constants or other components of the reactions at present. Nor is the agreement in form between the "experimental" and "theoretical" $G_A^{Na}$ curve in any way conclusive for the occurrence of the mechanism outlined above. There are certainly many other types of processes which may lead to similar results. However, the above treatment is justified by the fact that a semiempirical expression is obtained which seems to describe a property of frog skin approximately correctly, cf. below.

With the mechanism outlined above in mind, it is easy to imagine that metabolic disturbances or agents which interfere with the cyclic reaction may depress the partial conductance of Na ions by way of the carrier system towards zero (cf. also p. 50).

The resistance for diffusion through the tissues of the skin to the active membrane has not been considered, though it may be great enough to be of importance. Osmotic swelling of the epithelial cells might change this diffusion resistance, for instance, but some experiments made with isotonic sucrose on the outside

(LINDERHOLM 1952, foot-note p. 80) seem to indicate that such a change cannot be of great importance.

### The New "Hypothetical Skin".

The hypothetical frog skin proposed by LINDERHOLM (1952) was not well founded on experimental facts. Information about the behaviour of $G_A^{Na}$ and $\varphi_A^{Na}$ at various $c_1$ was especially scanty. The experiments here contribute a little more information. They allow the derivation of an expression for the variation of $G_A^{Na}$ with respect to $c_1$, which agrees satisfactorily with the $G_A^{Na}$-values estimated from the experiments. It deviates from the first rough assumption of a constant $G_A^{Na}$. The earlier assumption of a constant $\varphi_A^{Na}$ seems to be sustained by the experiments, however.

The new "hypothetical skin" may then be presented as follows: The partial conductances of the passive ions, mainly $G^{Cl}$ and $G_p^{Na}$, and their variation with $c_1$ are approximately expressed by eqn. 12 and $G_A^{Na}$, the partial conductance of the actively transported ions, by eqn. 21. The variation of the total conductance $G$ between various skins seems to depend principally on a variation in the partial conductances of the passive ions (cf. LINDERHOLM 1953). The transport potential of the sodium pump, $\varphi_A^{Na}$, has a constant value and is independent of $c_1$.

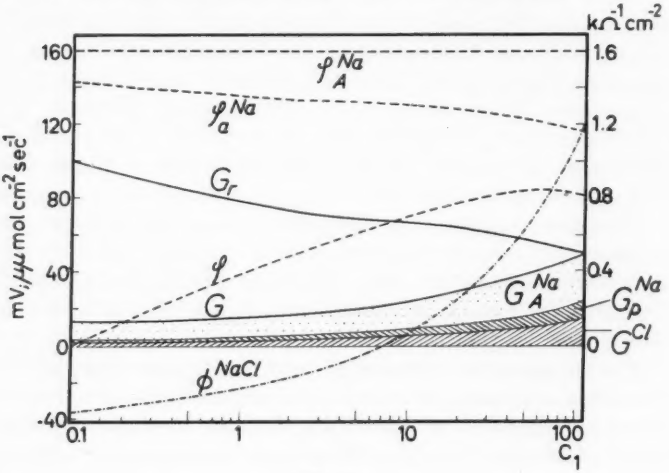


Fig. 5.

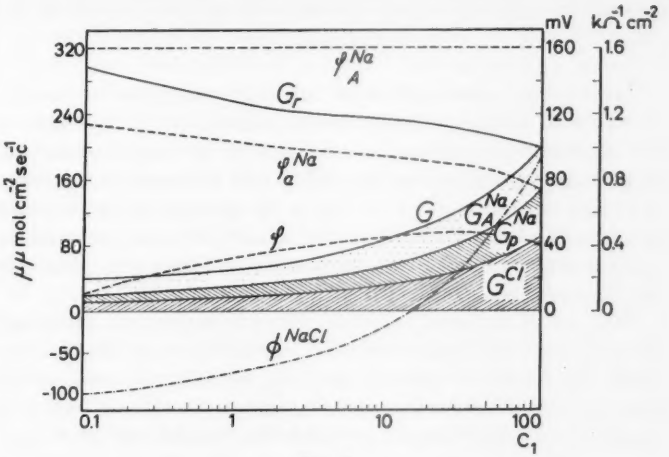


Fig. 6.

Figs. 5 and 6. These two figures represent two hypothetical frog skins. The total d. c. conductance $G$ when $c_1 = c_2$, *i. e.* with Ringer's solution on both sides, is 0.5 and 1.0 $k\Omega^{-1}$ $cm^{-2}$ respectively. The partial conductance of the actively transported Na ions is calculated from eqn. 21, and is represented by the height of the dotted area. The remaining part of $G$ is contributed by the partial conductance of Cl ions, $G^{Cl}$, and of passive Na ions, $G_p^{Na}$, so that $G_p^{Na} = 0.66\ G^{Cl}$. $G^{Cl}$ for lower $c_1$ is obtained from eqn. 12. The actively transported Na ions are exposed to an active force of 160 mV inwards through the skin. The rest of the parameters are then obtained from the equations in the theoretical part.

The hypothetical skin of low total conductance (Fig. 5) agrees well with the present experiments, as well as with some experiments in an earlier paper (LINDERHOLM 1952) for which $c_1$ was about 7 mM.

The hypothetical skin with higher total conductance (Fig. 6) is such that the values for $c_1 = c_2$ agree with the mean values of the earlier experiments reported by LINDERHOLM (1952 and 1953), where the skins were bathed with Ringer's solution on either side (mean $G = 1.0$ $k\Omega^{-1}$ $cm^{-2}$). The maximum $\varphi$ is at a lower $c_1$ in the latter case.

With these assumptions, the behaviour of frog skin for varying $c_1$ may be calculated, and two cases are worked out in Figs. 5 and 6. The constants were chosen with some regard to the results obtained in earlier experiments (see legend to Figs. 5 and 6). As will be seen, the agreement with experiments is satisfactory. Combined with the equations given by LINDERHOLM (1953), the above-mentioned set of equations describe the behaviour of frog skin under the actual experimental conditions, and with the help of a few measured parameters the others may be calculated with fair accuracy.

## Summary.

Experiments were performed to study the effect of various dilutions of Ringer's solution on the outside (1) of frog skin on the electrical potential, the electrical d. c. conductance, the permeability of the skin to Na and Cl ions as measured by means of radioactive isotopes (from which the partial conductances of the respective ions were estimated), the active transport potential of Na ions, and on the transport of NaCl through the skin. The inside of the skin was bathed with Ringer's solution.

Frog skin is supposed to behave like a homogeneous, uncharged (and "passive") membrane for Cl ions and part of the Na ions. Other Na ions pass through the skin by way of some specific carrier system that facilitates their passage through the skin both inwards and outwards and in which the Na ions are acted upon by an active transport force. This force tends to drive them inwards through the skin but may be counteracted (or helped) by electrochemical potential differences for Na ions across the skin. The equations used to estimate the different parameters of the skin from the experimental data were derived on these assumptions.

The concentration of the outside solution, $c_1$, was varied from about 0.4 mM to that of Ringer's solution. The results indicate that in this range it is possible to apply the equations and that they describe the behaviour of frog skin rather accurately. The partial conductance of Cl ions was found to decrease more with decreasing $c_1$ than would be expected for a homogeneous uncharged membrane, but this deviation may be due to the experimental procedure and a change in the living skin with time. The partial conductance of Na ions, on the contrary, decreases less than would be expected for diffusion through a homogeneous, uncharged membrane. It is discussed whether the results might be obtained with a negatively charged membrane, but some observations indicate that the "charge" is active only on Na ions, *i. e.* has the character of specific carrier.

The active transport potential of Na ions through the skin increases at low $c_1$, as might be expected if some of the Na ions were passive, *i. e.* not influenced by the active transport force. The conductance associated with the passive Na ions is assumed to be the same fraction of the partial conductance of Cl ions as

the mobility of Na ions is of that of Cl ions in water. An observation which seems to sustain this assumption is that when the sodium pump was inhibited in some experiments with mersalyl, $CO_2$, and dinitrophenol, the partial conductance for Na ions decreased and approached this fraction of the partial conductance of Cl ions. The assumption makes it possible to estimate a "true transport potential of the sodium pump", which is found to be fairly independent of $c_1$.

The net transport of NaCl through the skin could be determined by two independent methods, in some $c_1$ ranges at least. The good agreement indicates the essential correctness of the treatment.

The results are discussed, and some facts are put forward which makes it reasonable to regard the skin as a membrane where the essential diffusion resistance is due to a very thin membrane — perhaps the basement membrane — in which the active transport may also take place.

The nature of the sodium pump is discussed. Starting with a reaction scheme of a type similar to those proposed by other authors for ionic transport systems, an expression is derived for the conductance of the carrier system which, with properly chosen constants, gives a good description of the variation of the conductance of the carrier system with variation of $c_1$.

The features of a "hypothetical skin" are outlined. A set of equations derived in this and in an earlier investigation relates the various parameters of the isolated frog skin.

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## List of Some Symbols Used.

$a$ activity

B a chemical component

$c$ concentration

$F$ Faraday (96,500 coulombs)

$G$ electrical d. c. conductance

$G^{Cl}$ and $G_a^{Na}$ partial conductance of Cl and Na ions respectively

$G_{a\,I}^{Na}$ and $G_{a\,II}^{Na}$ partial conductance of Na ions calculated from eqns. 5 and 6 respectively

$G_A^{Na}$ and $G_p^{Na}$, see below under subscripts, $(G_a^{Na} = G_A^{Na} + G_p^{Na})$

M molar (moles/liter)

$R$ gas constant

$T$ absolute temperature

$\Phi$ net flux (positive in direction from solution 1 to solution 2)

$-\Phi_2$ total flux from solution 2 to solution 1.

$\varphi$ electrical potential $(\varphi_2 - \varphi_1 = \varphi)$

$\varphi_a^{Na}$ active transport potential of the skin for Na ions

$\varphi_A^{Na}$ "true active transport potential of the sodium pump"

$\xi$ is defined by eqn. 4

*Superscripts* refer to component species

*Subscripts* refer to solutions on either side of the membrane (skin) or to actively or passively transported ions as follows:

1 to the solution of the epithelial side of the skin (solution 1)

2 to the solution on the corium side (solution 2)

*a* to an actively transported component (Na ions)

*A* to Na ions which are transported actively through the sodium pump

*p* to Na ions which diffuse passively through the skin.

Other symbols are defined in connection with the equations.

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# Relationship between Low Values of Alveolar Carbon Monoxide Concentration and Carboxyhemoglobin Percentage in Human Blood.

By

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A simplified method for the determination of the total amount of hemoglobin and total blood volume, by administration of CO, has recently been described (SJÖSTRAND 1948 a and b). In this method, the alveolar CO concentration is first measured, using the CO analyser of LINDELÖV and SJÖSTRAND; a small amount of CO is then administered to the closed respiratory system, and after equilibrium has been reached between alveolar air and blood, the alveolar CO concentration is again determined. The total amount of hemoglobin in the body is calculated from the increase in alveolar CO concentration, produced by the known volume of administered CO, assuming a simple relationship between these low values of alveolar CO concentration and COHb percentage in blood (below 6 % COHb), as predicted by HALDANE's equation, $\frac{[COHb]}{[O_2Hb]} = M \cdot \frac{pCO}{pO_2}$. Duplicate determinations have shown an experimental error of about 4 % (ÅSTRAND 1952, SJÖSTRAND 1953). Measured in this way, the total amount of hemoglobin is closely correlated to body weight in children (KARLBERG and LIND 1953), total heart volume (KJELLBERG, LÖNROTH, RUDHE and SJÖSTRAND 1951), and maximum oxygen intake during muscular work (ÅSTRAND 1952). The method shows

good correspondence with other methods for blood volume determination (see SJÖSTRAND 1953).

Objections have, however, been raised to the method. It has been questioned (DARLING 1950) whether proportionality between alveolar CO concentration and COHb percentage in blood really exists within the stated range, and it has also been suggested that even if this is true in the one individual, the proportionality constant of the relationship may vary considerably between different individuals (DAHLSTRÖM 1953). These two problems have already been investigated by SIÖSTEEN and SJÖSTRAND (1951), using a modified ferricyanide method for COHb determination. Within a certain scatter, the percentage of COHb in blood was found to vary, as predicted, with alveolar CO concentration in 26 subjects. In three of these, the range of approximately direct proportionality was established by fractional administration of several small volumes of CO. The scatter of the values reflected the experimental errors involved in the determinations but between individuals small variations of the proportionality constant may have been present, increasing the scatter. If, in fact, there is a variation of considerable magnitude of the factors which determine the proportionality constant (equilibrium between alveolar air and blood, affinity of hemoglobin for CO), its effect on the CO-load method of determination of total amount of hemoglobin would obviously have to be assessed. One could only expect to obtain definite evidence on this point by using a more accurate method for COHb determination, than has hitherto been used.

The object of the present investigation has been to obtain additional data concerning the relationship between alveolar CO concentration and COHb percentage in the blood, by using three different methods for COHb determination, including that used by DAHLSTRÖM (1953). The result gives information about the proportionality constant of the relationship, its absolute magnitude, its variation with differing alveolar CO concentrations, and its variation between different individuals.

## Methods.

*Procedure.* For the determination of CO in gas, and for COHb in blood, samples were taken during the procedure of a routine determination of the total amount of hemoglobin (for details, see SJÖSTRAND

1948 b). The subject breathes into a closed system containing 100 % $O_2$, equipped with a $CO_2$ filter and an $O_2$ reservoir of approximately 5 liters volume (rubber bag). After fifteen minutes the rubber bag is removed for CO analysis (giving the initial value of alveolar CO concentration), and replaced by a second bag containing $O_2$. At this moment the first blood sample was taken, under paraffin oil, usually from an antecubital vein but in a few cases from a brachial artery. A known amount of CO is now added to the system, and fifteen minutes later the second bag is removed for CO analysis (giving the second value of alveolar CO concentration), and replaced by a third bag containing $O_2$. After fifteen minutes the second blood sample was taken, and the procedure terminated. The third bag was also analysed for CO content, giving the third value of alveolar CO concentration. The third value usually differs slightly from the second one, due partly to a more complete equilibrium (saturation of myoglobin, cf. SJÖSTRAND 1948 b), and partly to the small loss of CO to the third bag (about 0.5—1.0 ml, compared with an injected amount of 20—30 ml CO). The bags are analysed for $O_2$ content; leakage into the closed system is signalled by decreasing $O_2$ concentration, whereas leakage out from the system cannot be detected in this way. A closely fitting mouthpiece and continuous supervision of the subject is needed therefore to prevent leakage outwards.

*Analytical methods.* The gas samples were analysed for CO content by the method of SJÖSTRAND (1944, 1948 a), using the CO analyser of LINDELÖV and SJÖSTRAND. It was assumed that the analysed gas sample was representative of mean alveolar composition. The CO concentration was obtained in terms of volume per volume dry gas. The alveolar pCO was calculated, corresponding to normal barometric pressure and allowing for an alveolar $pH_2O$ of 48 mm Hg and an alveolar $pCO_2$ of 40 mm Hg.

Each blood sample was always analysed in duplicate for CO content by three methods. In addition, the CO capacity (CO content after full saturation with CO) was determined in duplicate by these three methods, in one blood sample from each subject, in order to allow the calculation of relative COHb percentage. The methods were:

(A) the method of HORVATH and ROUGHTON (1943), which uses the manometric van Slyke apparatus;

(B) the method of SIÖSTEEN and SJÖSTRAND (1951), which uses $K_3Fe(CN)_6$ to liberate CO bound to hemoglobin; ten liters of CO-free air are bubbled through the blood sample, after chemical treatment, and this air is collected in two consecutive rubber bags; usually, the second bag contained small amounts of CO;

(C) a modification of the method of SIÖSTEEN and SJÖSTRAND, introduced by DAHLSTRÖM (1953) after WENNESLAND (1940); 10 % $H_2SO_4$ is used to liberate CO from hemoglobin instead of $K_3Fe(CN)_6$ but the other procedure is the same; in this case, the second rubber bag did not contain measurable amounts of CO.

*Material.* The material investigated consisted of 33 male and 27 female subjects, including one anemic patient, nine polycytemic

patients and eighteen patients with heart disease; 22 subjects were smokers.

## Results.

A comparison was first made between the three methods for determination of CO concentration in blood. Blood samples were taken from five subjects, not deliberately exposed to CO, and analysed by triple determination by each of the three methods. The blood samples were found to contain small amounts of CO, mean value 0.15 ml CO per 100 ml blood (method A), 0.19 (method B) and 0.25 (method C). The difference between the methods (calculated from the individual differences, as in the following) was statistically highly significant ($P<0.001$); therefore, at these low CO concentrations, method B gives higher values than method A, and C higher than B. The difference was still present if the CO content was expressed as COHb percentage but it was not identical; the reason being that when the total CO capacity of a blood sample was measured, method C gave higher values, method B lower values than method A. For example, in nine subjects the CO content at full saturation was 19.0 ml CO per 100 ml blood (method A) and 17.5 (method B), while in nineteen other subjects it was 19.6 (A) and 20.9 (C). The difference between A and B was statistically probably significant ($P = 0.05$), between A and C highly significant ($P < 0.001$). If these mean values for CO capacity are used to calculate COHb percentage for the first five subjects, the result is 0.79 % COHb (method A), 1.09 % (B) and 1.20 % (C). There is, therefore, a significant difference between the methods, the importance of which will be further discussed below.

The relationship between alveolar pCO and COHb percentage in blood was investigated in 55 subjects, using method A alone in 27, A and B in nine, and A and C in nineteen of these. Fig. 1 shows the result of the determinations. In the Figure, a full line drawn from origo indicates the relationship (approximately direct within this range) which is given by HALDANE's equation. As in the course of an experiment the alveolar $pO_2$ normally showed some variation, ranging between 95 % and 90 % $O_2$ in the inspired air, the alveolar pCO was corrected, in order to permit comparison of the different values, to correspond to exactly 90 % $O_2$ (alveolar $pO_2 = 605$ mm Hg) in the Figure, using HALDANE's equation.

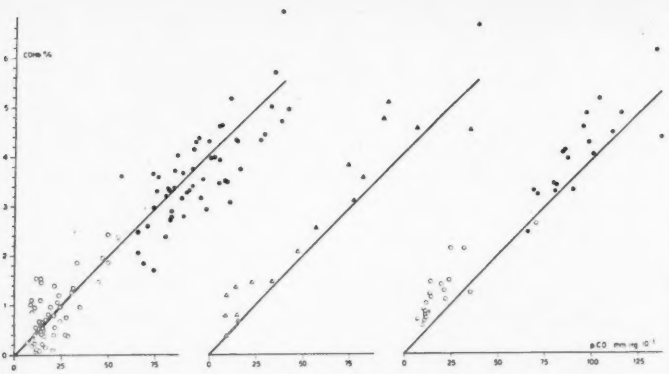

Fig. 1. Relationship between alveolar pCO (abscissae) and COHb percentage in blood as determined by, from left to right, methods A, B and C (ordinates) in a total number of 55 subjects before (open symbols) and after (filled symbols), CO administration. A line from origo indicates the relationship when M (Haldane's equation) has a value of 245.

Table 1 shows the mean values of COHb percentage, calculated from the mean values of alveolar pCO by the use of HALDANE's equation, with a value of 245 for M. This value for M was chosen as it is the mean value obtained by method A for COHb determination, and therefore considered to be representative (see Table 3).

Before CO administration (Fig. 1, Table 1), the COHb percentage in blood was low, with mean values about 1 % (compare above). There was a large variation within the material, however, and the values for smokers were considerably higher than for non-smokers. For this reason, the distribution curve of the values was markedly skew, as is apparent from the large standard deviations. Method B was in this case (cf. above) found to give values approximately identical with those of method A ($P = 0.9$). Method C gave considerably higher values, with a difference from A which is statistically significant

$$(0.01 > P > 0.001).$$

After CO administration, the COHb percentage increased to about 4 %, with a considerable variation of the material. Method B again gave values approximately identical with those of method A, with a difference which is not statistically significant ($P = 0.9$);

**Table 1.**

*COHb percentage, calculated from alveolar pCO ($M = 245$) and determined by methods A, B and C.*

| | No. of subjects | Calculated from alveolar pCO | Determined by method | | |
|---|---|---|---|---|---|
| | | | A | B | C |
| *Before CO administration* | | | | | |
| Mean | 9 | 0.76 | 1.14 | 1.15 | — |
| S. D. | | 0.53 | 0.69 | 0.52 | — |
| Mean | 19 | 0.75 | 0.92 | — | 1.24 |
| S. D. | | 0.56 | 0.63 | — | 0.55 |
| Mean | 27 | 0.96 | 0.73 | — | — |
| S. D. | | 0.59 | 0.69 | — | — |
| *After CO administration* | | | | | |
| Mean | 9 | 3.72 | 4.30 | 4.33 | — |
| S. D. | | 1.13 | 1.28 | 1.27 | — |
| Mean | 19 | 3.62 | 3.73 | — | 4.07 |
| S. D. | | 0.77 | 0.93 | — | 0.86 |
| Mean | 27 | 4.02 | 3.45 | — | — |
| S. D. | | 0.83 | 0.73 | — | — |
| *Increase after CO administration* | | | | | |
| Mean | 9 | 2.96 | 3.16 | 3.18 | — |
| S. D. | | 0.90 | 1.25 | 1.06 | — |
| Mean | 19 | 2.87 | 2.81 | — | 2.83 |
| S. D. | | 0.75 | 0.81 | — | 0.70 |
| Mean | 27 | 3.06 | 2.72 | — | — |
| S. D. | | 0.79 | 0.86 | — | — |

**Table 2.**

*Value of M (Haldane's equation) calculated from increase of COHb percentage in relation to increase of pCO after CO administration. Methods A, B and C for COHb determination, 55 subjects.*

| | A | B | C |
|---|---|---|---|
| No. of subjects | 55 | 9 | 19 |
| Mean | $228 \pm 6$ | $260 \pm 20$ | $231 \pm 7$ |
| S. D. | $45 \pm 5$ | $59 \pm 14$ | $31 \pm 5$ |

C gave higher values than A, with a significant difference ($P = 0.01$).

The increase in COHb percentage caused by CO administration was about 3 %. The mean increase was practically identical by all three methods, as is apparent from Fig. 2. Statistical evaluation

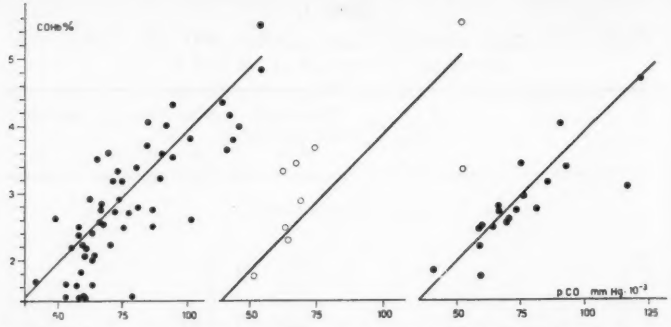


Fig. 2. Relationship between increase of alveolar pCO (abscissae) and increase of COHb percentage in blood as determined by methods A, B and C, from left to right (ordinates) caused by CO administration. A line from origo indicates the relationship when M (Haldane's equation) has a value of 245.

showed good agreement between methods B and A ($P = 0.6$) and C and A ($P = 0.8$).

It is apparent, therefore, that the difference in COHb values between the three methods for COHb determination is of approximately constant magnitude within the relevant range of concentrations. This observation can be interpreted in different ways. The most probable explanation is that method B gives a small, and C a larger, constant blank value. This should originate from the hemoglobin of the blood sample, as earlier plasma has been found not to give a blank value (method B, SIÖSTEEN and SJÖSTRAND 1951). Carbon monoxide is formed during the decomposition of hemoglobin, more rapidly at low values of pH (SJÖSTRAND 1951). Method C, in which 10 % $H_2SO_4$ is added to the blood sample, also gives the highest values of the methods. A probable explanation for the discrepancy between the methods is therefore, that during the chemical treatment of the blood sample a gas is formed which is either CO, or some other gas which behaves like CO in the CO analyser.

The proportionality constant M of HALDANE's equation was calculated for each individual set of values of alveolar pCO and COHb percentage. Fig. 3 shows the relation between alveolar pCO and M, obtained by three different methods of COHb determination, including the values before and after CO administration. Mean values relating to Fig. 3 are shown in Table 3. By taking successive media, a smooth regression line of curved

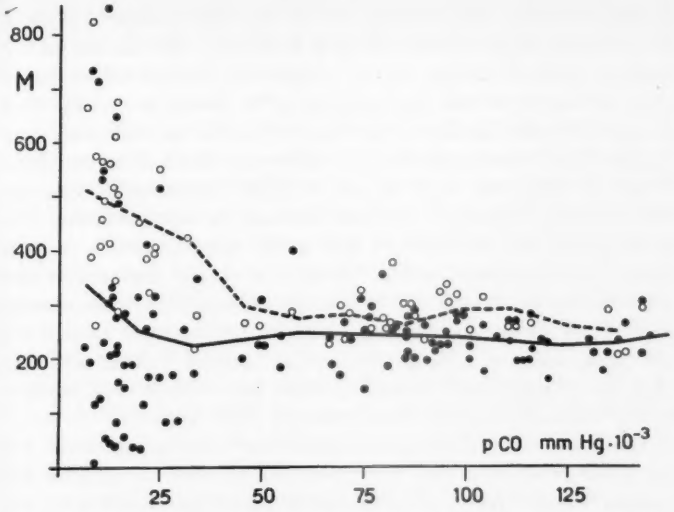


Fig. 3. Relationship between alveolar pCO at an alveolar $pO_2$ of 605 mm Hg (abscissa) and the proportionality constant of Haldane's equation (ordinates). Method A: filled circles and full line; methods B and C: open circles and dashed line.

**Table 3.**

*Value of M (Haldane's equation) at different values of alveolar pCO (alveolar $pO_2$ = 605 mm Hg), obtained before and after CO administration in 55 subjects.*

| Alveolar pCO mm Hg $10^{-3}$ | Method A | | | Methods B + C | | |
|---|---|---|---|---|---|---|
| | No. of subjects | Mean | S. D. | No. of subjects | Mean | S. D. |
| 0— 13 | 15 | 336 | 276 | 11 | 512 | 151 |
| 13— 26 | 26 | 227 | 143 | 12 | 456 | 120 |
| 26— 39 | 7 | 197 | 97 | 3 | 309 | |
| 39— 52 | 3 | 245 | | 1 | 264 | |
| 52— 65 | 4 | 262 | | 1 | 283 | |
| 65— 78 | 10 | 236 | 56 | 6 | 274 | 37 |
| 78— 91 | 13 | 243 | 47 | 8 | 287 | 43 |
| 91—104 | 10 | 238 | 30 | 6 | 301 | 33 |
| 104—117 | 9 | 234 | 38 | 4 | 273 | |
| 117—130 | 5 | 206 | | — | — | |
| 130—143 | 7 | 227 | 55 | 4 | 250 | |
| 143—156 | 1 | 239 | | — | — | |
| 0—156 | 110 | 245 | | 56 | 364 | |

appearance was obtained (Fig. 3). At very low values of pCO M shows considerable scatter, ranging from 0 to 820. As shown by Table 3, methods B and C give considerably higher values for M than method A, within this range of pCO. This is in consequence of the difference between the three methods, as discussed previously. If it is assumed that this difference depends on methods B and C giving too high values of COHb percentage, then the values for M obtained by method A should be representative. The mean value by this method before CO administration is 254, after CO administration 237. There is a steady decrease of the mean values of M, obtained by methods B and C, with increasing pCO (Table 3) but for method A this is not the case. Except for the initial value of 336, M obtained by method A shows an irregular, and apparently random, variation without an obvious trend, with an over-all mean value of 245 (Table 3).

The increase of COHb percentage after CO administration has a regular relation to the corresponding increase of alveolar CO concentration. When the coefficient M of this relation is calculated for method A (Table 2) a mean value of $228 \pm 6$ is obtained, with a standard deviation of $45 \pm 4$. In view of the larger variation of M shown in Fig. 3, this is a relatively small scatter. It is important to note that this value of M, signifying the capacity of hemoglobin for binding extra administered CO, is the relevant one for the calculations entailed in the indirect method for determination of the total amount of hemoglobin, as described by SJÖSTRAND (1948 b).

It now remains to consider whether the variation of M shown in Fig. 3 and Tables 2 and 3 is simply due to large errors of measurement or if, in addition, they reflect considerable real variations between individuals of those biological factors which influence M, such as the rate of equilibration between alveolar air and blood, or the affinity of hemoglobin for CO. The errors of measurement which influence the obtained values of M are the following.

(i) The analysis of CO content of a gas sample by the CO analyser has been found earlier to have an experimental error of less than 2 % of the mean value, except at very low CO concentrations, when it may be slightly greater (SJÖSTRAND 1948 a, SIÖSTEEN and SJÖSTRAND 1951). This error is of relatively little importance in the determination of M.

(ii) In analysis with the CO analyser, the unknown sample is

always compared with a known standard gas (usually 0.01 % CO in air). A large amount of standard gas is always kept in store at the laboratory and therefore, for the period of this investigation, there was hardly any fluctuation in the composition of the standard. The absolute value of the calibration may not be absolutely correct, however, and this constant error is difficult to estimate. The error would be of importance when absolute values of CO concentration are considered but it would cancel out for relative values; thus, a value of M obtained by method A would carry this error but a value obtained by methods B or C would not, as in the latter case the determination of the gas sample, as well as of the blood sample, was made in reference to the standard gas.

(iii) The error in measuring COHb percentage in blood is considerable. For method B, the error had been found earlier to be 6.4 % of the mean, for values between 0 % and 2 % COHb, and 4 % for 2—4 % COHb (SIÖSTEEN and SJÖSTRAND 1951). In the present investigation, calculated in the usual way from duplicate determinations, the error of method B was larger, being 10 % and 6 % of the mean for the two respective ranges of COHb concentration, while for method A it was 19 % and 9 %, and for method C 16 % and 5 %.

In the calculation of M for the increase of COHb in relation to the increase of alveolar CO concentration (mean value $228 \pm 6$, S. D. $43 \pm 4$, see Table 2), the afore-mentioned errors are involved as well as other analytical errors. An estimation indicates that M should be expected to have an experimental error of considerably more than 12 % of the mean.

The actual value of the experimental error of M was determined, in a series of 20 subjects, by duplicate determinations in each subject, with an interval of one or two days. The result is shown in Table 4. The mean values of M are lower than for the rest of the material investigated, and lower in the first, than in the second determination. The reason for these differences is unknown. The error in a single determination of M, calculated in the usual way from the differences between duplicate determinations, is shown in the Table. Before CO administration this error is 78, and the S. D. of the whole group of subjects is 83 and 101 (first and second determinations respectively). After CO administration the error is 30, and the S. D. of the group 32 and 38. For values of M relating to the increase of pCO and COHb %,

**Table 4.**

*Value of M (Haldane's equation) obtained from duplicate determinations (interval of one or two days) in 20 subjects. Method A for COHb determination.*

| | No. of subjects | Mean $\pm e_M$ | S. D. $\pm e_{S.D.}$ | Error in single determination |
|---|---|---|---|---|
| Before CO administration | | | | |
| first determination ..... | 20 | 173 ± 19 | 83 ± 13 | 78 (45 %) |
| second » ..... | 20 | 205 ± 23 | 101 ± 16 | (38 %) |
| After CO administration | | | | |
| first determination ..... | 20 | 206 ± 7 | 32 ± 5 | (15 %) |
| second » ..... | 20 | 218 ± 9 | 38 ± 6 | 30 (14 %) |
| Increase after CO administration | | | | |
| first determination ..... | 20 | 201 ± 11 | 49 ± 8 | 46 (23 %) |
| second » ..... | 20 | 224 ± 10 | 43 ± 7 | (20 %) |

the error is 46 and the S. D. is 49 and 43. The whole variation of M within this group of 20 subjects is therefore allowed for by the experimental error involved in the determination.

## Discussion.

In the present investigation, duplicate determinations of the total amount of hemoglobin were made in 36 subjects. In 27 non-smokers the experimental error was 3.9 % of the mean; in nine smokers the error was considerably larger, about 7.6 %. In two earlier reports the experimental error of the method had been found to be just below 4 % (ÅSTRAND 1952, SJÖSTRAND 1953). The total experimental error includes the error in determining the increase of alveolar CO concentration due to CO administration, the error in measuring the volume of CO which is administered, and the possible error in leakage out from the closed respiratory system (see Methods); but also the biological variation of the subject, including changes in the equilibration process, changes in the affinity of hemoglobin for CO and changes in the amount of hemoglobin and myoglobin of the body, during the period between the duplicate determinations. Obviously, the biological variation within the single individual must be very slight, if any, as the errors in measurement will easily account

for the total experimental error. The proportionality constant M, therefore does not vary noticeably in the one individual. But is there a large variation of M between individuals, as suggested by DAHLSTRÖM (1953)? In the present investigation, there were variations of M between individuals of approximately the same order of magnitude as in DAHLSTRÖM's material. The error in measurement of M was, however, equally as large as the standard deviation in a group of 20 subjects; therefore, the conclusion can be drawn that the observed variation of M is largely produced by inaccuracy of method (inaccuracy of methods for direct determination of COHb). A small biological variation of M cannot, of course, be excluded until a more accurate method for COHb determination is available; but so far no evidence for the existence of such a variation has been produced.

There is some uncertainty in deciding which absolute value of M should be regarded as the most representative one. SENDROY, LIU and VAN SLYKE (1929) reported a value of $210 \pm 3$ and FORBES, SARGENT and ROUGHTON (1945) found that a CO concentration of the inspired air of 0.01 % produces, at an alveolar $pO_2$ of 100 mm Hg, a COHb percentage in blood of 14 %. The corresponding value of M depends, according to HALDANE's equation, on the $O_2Hb$ percentage and the alveolar pCO; if these are chosen as 86 %—83 %, at an alveolar pCO of 0.0672 mm Hg, the value of M will be 243—251. In the present investigation, similar values were found. Values obtained by methods B or C for COHb determination probably entail a systematic error (see under Results). By method A, the mean value for all concentrations of alveolar pCO was 245 (Table 3), which may be regarded as fairly representative. The mean value of M which relates increase of pCO to increase of COHb after CO administration was lower, $228 \pm 6$, although not significantly different. In conclusion, therefore, the value of M seems to be approximately constant at values of COHb percentage ranging from 0 % to 14 %, the deviation at values below 1 % (see Fig. 3) not being statistically significant.

## Summary.

1. Alveolar CO concentration and COHb percentage in blood were determined in 55 subjects before and after exposure to low concentrations of CO.

2. Percentage of COHb in blood was found to increase in, approximately, direct relationship with increasing values of alveolar pCO within the investigated range (below 6 % COHb), as predicted by HALDANE's equation.

3. The mean value of M in HALDANE's equation for the whole range of alveolar CO concentrations was 245.

4. When a small amount of CO was administered to a subject, the increase of COHb percentage was related to the increase of alveolar CO concentration by the value of M of $228 \pm 6$, S. D. $45 \pm 5$.

5. The variation of M in the material investigated could be explained as produced wholly by errors in measurement. Therefore, no real variation of M between different subjects could be established.

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# Alimentary Production of Gallstones in Hamsters. III.

By

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Previous communications (DAM and CHRISTENSEN 1952, CHRISTENSEN, DAM and PRANGE 1952) described the production of gallstones in hamsters by purely dietetic means and, further, that gallstone formation could be prevented completely or partially by incorporation of certain cereal grains into the diet.

The present paper concerns the gallstone preventing activity of several cereals and grains, yeast and some other natural food materials at varying dietary levels, certain fractions thereof and some of the components of an artificial diet which has low gallstone producing properties.

## Experimental.

The procedure for producing and recording the symptom was the same as described in communication II, each group of hamsters consisted of 9 to 10 animals, 4 or 5 of each sex. The basal diet was no. 295 (communication II). The seeds to be tested were all ground before incorporation into the diet.

## Results and discussion.

The results are presented in condensed form in table 1.

In communication II the seeds of durra (Sorghum vulgare) were found to be more efficient than the cereals tested (whole wheat and rolled oats). However, several shipments of durra gave somewhat varying results, wherefore we decided to test a series of other seeds.

At the 72.3 % level (all the sucrose replaced by the seeds to be tested) hempseed, soybeans and white rice gave good protection.

**Table 1.** (section A).

| Group no. | Diet no. | Characteristics of diet | Duration of experiment days | Incidence of gallstones compared with control group[1] per cent |
|---|---|---|---|---|
| 42 | 314 | 72.3 % hempseed | 58 | 0 |
| 53 | 314 | » | 70 | 0 |
| 81 | 314 | » | 65 | 12 |
| 95 | 314 | » | 63 | 0 |
| 43 | 315 | 72.3 % soybeans | 58 | 11 |
| 148 | 315 | » | 61 | 0 |
| 78 | 343 | 72.3 % polished rice | 61 | 11 |
| 54 | 323 | 36.15 % hempseed | 70 | 10 |
| 72 | 337 | 36.15 % linseed | 61 | 0 |
| 73 | 338 | 36.15 % rapeseed | 61 | 0 |
| 74 | 339 | 36.15 % sunflower seed (large fibers removed) | 61 | 20 |
| 76 | 341 | 36.15 % peeled peanuts | 61 | 0 |
| 79 | 344 | 36.15 % yellow peas | 61 | 11 |
| 75 | 340 | 36.15 % yellow corn | 61 | 81 |
| 77 | 342 | 36.15 % millet | 61 | 78 |
| 89 | 352 | 18.1 % soybeans | 65 | 0 |
| 126 | 352 | » | 57 | 0 |
| 129 | 372 | 18.1 % soybean flour | 57 | 11 |
| 132 | 375 | 18.1 % Fleischmann yeast (type 2019) | 57 | 0 |
| 82 | 345 | 18.1 % hempseed | 65 | 63 |
| 83 | 346 | 18.1 % linseed | 65 | 50 |
| 84 | 347 | 18.1 % rapeseed | 65 | 25 |
| 85 | 348 | 18.1 % peeled peanuts | 65 | 50 |
| 86 | 349 | 18.1 % yellow peas | 65 | 37 |
| 87 | 350 | 18.1 % polished rice | 65 | 111 |
| 88 | 351 | 18.1 % whole wheat | 65 | 100 |
| 90 | 353 | 18.1 % rye | 65 | 100 |
| 91 | 354 | 18.1 % barley | 65 | 88 |
| 154 | 389 | 9 % soybeans | 61 | 79 |
| 152 | 387 | 9 % Fleischmann yeast (type 2019) | 61 | 79 |
| 155 | 390 | 4.5 % soybeans | 61 | 59 |
| 153 | 388 | 4.5 % Fleischmann yeast (type 2019) | 61 | 100 |
| 55 | 329 | 47.72 % ether extracted hempseed | 70 | 0 |
| 97 | 324 | » » » » | 63 | 0 |
| 61 | 329 | 24.1 % hempseed oil | 70 | 60 |
| 57 | 326 | 48.2 % wheat starch + 24.1 % hempseed oil | 70 | 64 |
| 56 | 325 | 63.9 % pressed hempseed | 70 | 10 |
| 102 | 357 | 3.6 % ash of hempseed | 63 | 60 |
| 103 | 358 | 5 % dried percolate (60 vol.% alcohol) of ether extracted hempseed | 63 | 50 |
| 104 | 359 | 47.7 % ether extracted percolated (60 vol.% alcohol) hempseed | 63 | 0 |
| 138 | 378 | 8 % soybean flour extract (30 vol.% alcohol) | 47 | 0 |
| 137 | 377 | 23.7 % extracted soybean flour (30 vol.% alcohol) | 47 | 0 |
| 159 | | 4 % water extracted Fleischmann yeast | 58 | 125 |
| 158 | | 14 % » » » » | 58 | 0 |
| 157 | | 4 % water extract of Fleischmann yeast | 58 | 125 |

[1] $\frac{\text{per cent of animals with gallstones in group}}{\text{per cent of animals with gallstones in control group}} \times 100$

The incidence in the control group was, in most cases, between 80 and 100 per cent.

At the 36.15 % level (half of the sucrose replaced by the seed to be tested) hempseed, linseed, rapeseed, sunflower seeds, peanuts without shells, and yellow peas gave good or fairly good protection whereas yellow maize and millet failed to protect or, at least, gave poor protection.

At the 18.1 % level, good protection was obtained with soybeans, soybean flour and Fleischmann Yeast (type 2019), whereas hempseed, linseed, rapeseed, peanuts, yellow peas, white rice, wheat, rye, and barley failed more or less.

At the 9 and 4.5 % levels also soybeans and Fleischmann yeast failed.

Before the superiority of soybeans and yeast was found, an attempt was made to locate the activity of hempseed in certain easily obtainable fractions, viz., the defatted seed, the oil and the ash. Ether-extracted hempseed given at the level of 47.7 %, corresponding to 72.3 % unextracted seed, was found in two experiments to give full protection. Hempseed oil, obtained in the laboratory by pressing, at a level of 24.1 %, corresponding to 72.3 % hempseed, gave slight protection in two experiments, one with sucrose, the other with starch as carbohydrate. The ash given at the level of 3.5 %, corresponding to 72.3 % hempseed, also gave a slight protection only. A percolate, prepared with 60 vol.% ethyl alcohol from ether extracted hempseed fed at the level of 5 %, corresponding to 72.3 % of hempseed, gave a protection of 50 %. The percolated hempseed fed at the level of 47 %, corresponding to 72.3 % hempseed, gave full protection. Thus, at least the main portion of the protective principle in hempseed is present in the combustible ether insoluble fraction which is not, or not easily, soluble in 60 vol.% alcohol. The fact that the nearly fat-free soybean flour protects emphasizes further that the main active principle is not fat-soluble.

An extract prepared by boiling soybean flour with 30 vol.% ethyl alcohol for 10 minutes and repetition of this procedure once gave full protection when fed at the level of 8 %, corresponding to 36.5 % soybean flour. The portion which remained undissolved by this extraction procedure also gave full protection at a level of 23.7 %, corresponding to 36 % soybean flour. Therefore it seems that some of the active principle from soybean flour can be extracted with boiling 30 % alcohol but, at least under the conditions chosen, the extraction was incomplete.

An extract of Fleischmann yeast, prepared by boiling with

water 10 minutes, centrifuging and repeating the procedure once, failed to show any activity when fed at the level of 4 % dry matter, corresponding to 18 % untreated dry yeast, whereas the activity was found in the undissolved portion of the yeast fed at the level of 14.6 % dry matter, corresponding to 18 % of the untreated dry yeast. When fed at the level of 4 % the undissolved portion failed to protect. Thus, simple boiling with water is not sufficient to extract the active principle from dry yeast.

Along with the attempts to locate the active principle in fractions of the protective materials, the influence of a series of variations of the diet was tested, viz., those listed in *Table 1, section B.*

From these experiments it is seen that sitosterol failed to protect even though it might be thought to influence the symptom by inhibiting absorption of cholesterol from the intestine (PETERSON, 1951, PETERSON et al. 1952, 1953). Lecithin from egg-yolk and soybeans, as well as whole egg-yolk in the doses tested, gave no protection either. Variation of the content of choline chloride from 0 to 0.4 % was also ineffective. In the doses tested inositol, cystine and taurine were ineffective. Gum arabic and two kinds of beet sugar molasses were similarly without protective action.

One-half per cent cholesterol gave the same incidence of gallstones as the control group on diet no. 295, viz., in 7 out of 9 animals. Eighteen per cent Fleischmann yeast, type 2019, lowered the incidence on the cholesterol containing diet to 2 out of 9 animals. Increase of the content of lard from 2 % in the basal diet 295 ot 30 % resulted in a partial protection, the incidence being 50 % in the latter case.

Variation of the content of casein from 10 % to 15 % and 30 % gave no significant protection compared with the 20 % casein in the control group (basal diet 295). Inclusion of roughage (cellufluor extracted with ether) at the level of 10 % in the basal diet did not protect.

At this point it may be of interest to mention that two diets commonly used in this laboratory, viz., a commercial chicken mash (DAM and SØNDERGAARD, 1953), and a basal diet for hamsters no. 330 (table 2) did not give rise to gallstones in hamsters or at least to a very low degree only.

Certain experiments were carried out in order to find which features of this last mentioned diet were responsible for its pro-

**Table 1** (section B).

| Group no. | Diet no. | Characteristics of diet | Duration of experiment days | Incidence of gallstones compared with control group[1] per cent |
|---|---|---|---|---|
| 24 | 304 | 0.1 % sitosterol | 58 | 100 |
| 25 | 305 | 0.5 % » | 58 | 100 |
| 48 | 319 | 5 % egg lecithin | 58 | 101 |
| 47 | 318 | 8 % soya lecithin "60 % pure" | 58 | 101 |
| 46 | 317 | 10 % egg yolk | 58 | 90 |
| 49 | 320 | no choline chloride | 58 | 112 |
| 59 | 320 | » » » | 70 | 100 |
| 60 | 328 | 0.4 % choline chloride | 70 | 100 |
| 69 | 335 | 0.5 % inositol | 61 | 89 |
| 70 | 336 | 0.5 % cystine | 61 | 100 |
| 164 | 397 | 0.5 % taurine | 28 | 100 |
| 105 | 360 | 5 % gum arabic | 63 | [1] 100 |
| 106 | 361 | 5 % dried beet sugar molasses | 63 | [1] 100 |
| 107 | 362 | 5 % dried fermented molasses[2] | 63 | [1] 100 |
| 162 | 395 | 0.5 % cholesterol | 28 | 100 |
| 163 | 396 | 0.5 % cholesterol + 18.1 % Fleischmann yeast 2019 | 28 | 28 |
| 29 | 309 | 30 % lard | 58 | 50 |
| 51 | 322 | 10 % casein | 58 | 79 |
| 50 | 321 | 15 % casein | 58 | 101 |
| 58 | 327 | 30 % casein | 70 | 80 |
| 101 | 356 | 10 % ether extracted celluflour | 63 | 100 |
| 64 | | Commercial chicken mash | 61 | 0 |
| 98 | 330 | Artificial "nonlithogenic" | 63 | 0 |
| 125 | 330 | » » | 57 | 23 |
| 135 | 330 | » » | 63 | 0 |
| 136 | 376 | 330 with 5 % salt mixture instead of 4 % | 63 | 0 |
| 131 | 374 | 330 with vitamins A, D in lard | 57 | 0 |
| 140 | 380 | 330 with same vitamin and salt mixtures as in diet 295 | 63 | 38 |
| 130 | 373 | 330 with same vitamin mixture as diet 295 | 63 | 13 |
| 117 | 365 | 295 with vitamin mixture as in diet 330. | 60 | 90 |
| 118 | 366 | 295 with ascorbic acid as in diet 330 | 60 | 70 |
| 119 | 367 | 295 with folic acid as in diet 330 | 60 | 80 |
| 120 | 368 | 295 with PABA as in diet 330 | 60 | 70 |
| 127 | 364 | 295 with A, D-vitamins in peanut oil instead of in lard | 57 | 98 |
| 116 | 363 | 295 with same salt mixture as in diet 330 (4 %) | 60 | 50 |
| 128 | 371 | 295 with 4 % salt mixture instead of 5 % | 57 | 100 |
| 143 | 383 | 295 + copper sulfate as in diet 330 | 35 | [3] 60 |
| 144 | 383 | 295 with manganous sulfate as in diet 330 | 35 | [3] 200 |
| 142 | 382 | 295 + KI as in diet 330 | 35 | [3] 178 |

[1] only males.
[2] distillers spent wash (from beet sugar molasses).
[3] in this case, the control group showed only an incidence of 50 % gallstones.

| Group no. | Diet no. | Characteristics of diet | Duration of experiment days | Incidence of gallstones compared with control group[1] per cent |
|---|---|---|---|---|
| 150 | 385 | 295 with 1/3 the amount of copper sulfate as in 330 | 61 | 120 |
| 149 | 383 | 295 with copper sulfate as in diet 330. | 61 | 20 |
| 151 | 386 | 295 with 3 times the amount of copper sulfate as in diet 330 | 61 | 0 |

Table 2.

Diet 330.[1]

| | |
|---|---|
| Crude casein | 25.0 |
| Sucrose | 63.1 |
| Salt mixture[2] | 4.0 |
| Vitamin mixture[3] | 0.5 |
| Choline chloride | 0.4 |
| Lard | 7.0 |
| | 100.0 |

Vitamins A and $D_3$ were given in peanut oil twice weekly (25 I.U. A, 5.3 I.U. $D_3$ and 9 mg peanut oil per day, corresponding to the amounts of these vitamins received by each animal per day in diet 295, when the daily consumption of this diet is 4 grams.

[1] H. Granados, Acta Physiol. Scand. *24*, Supplementum 87, p. 33, 1951.

[2] Salt mixture no. 185. McCollum Simmond[4], supplemented with 0.131 % $CuSO_4$, $5H_2O$, 0.542 % $MnSO_4$, $4H_2O$ and 0.0131 % KI.

[3] The 0.5 g of vitamin mixture contained: biotin 20 $\mu$g, folic acid 400 $\mu$g, thiamine hydrochloride 5 mg, riboflavin 5 mg, pyridoxine hydrochloride 5 mg, calcium pantothenate 5 mg, nicotinic acid 7.5 mg, p-aminobenzoic acid 100 mg, inositol 100 mg, ascorbic acid 20 mg, vitamin K substitute (dicalcium salt of 2-methyl-1,4-naphtholhydroquinone diphosphate) 3 mg, dl-$\alpha$-tocopherol acetate 5 mg, and sucrose 244.080 mg.

Diet 295.

| | |
|---|---|
| Casein, crude | 20.0 |
| Sucrose | 72.3 |
| Salt mixture[1] | 5.0 |
| Vitamin mixture[2] | 0.5 |
| Choline chloride | 0.2 |
| Lard with vitamins A and D[3] | 2.0 |
| | 100.0 |

[1] Salt mixture no. 2, U. S. P. XIII.

[2] Biotin 50 $\mu$g, folic acid 50 $\mu$g, ascorbic acid 5 mg, thiamine hydrochloride 5 mg, riboflavin 5 mg, pyridoxine hydrochloride 5 mg, calcium pantothenate 5 mg, nicotinic acid 8 mg, inositol 15 mg, p-aminobenzoic acid 35 mg, vitamin K ("Synkavit", Roche) 3 mg + sugar up to 500 mg.

[3] 2.00 g contained 0.7 mg vitamin A palmitate ("Mywax 16", Distillation Products Industries, Rochester, N. Y.) and 0.667 mg of a solution of irradiated 7-dehydrocholesterol in soybean oil (Ferrosan A/S, Copenhagen), corresponding to 670 I.U. A and 133 I.U. $D_3$.

[4] J. Biol. Chem. 1918, *33*, 55.

tective effect. At first it was found that diet no. 330 did not become gallstone-producing (lithogenic) when the salt mixture was given at a level of 5 % instead of 4 %, or when vitamins A and D were incorporated into the lard instead of being given in peanut oil (as drops).

Simultaneous replacement of the vitamin and salt mixtures in diet 330 with those of diet 295 did not change diet 330 into an efficiently gallstone producing diet either, although a few animals in the group had gallstones. The same was found when the vitamin mixture and the salt mixture were changed separately. Therefore, the protective properties of diet no. 330 may be due to a combination of several features probably involving the levels of lard and casein also.

When in diet 295 the vitamin mixture was replaced by the vitamin mixture from diet 330 no significant protection resulted; slight or insignificant protection was obtained by increasing the amounts of ascorbic acid, folic acid or PABA in 295 up to the levels used in diet 330.

Administration of vitamins A and D in peanut oil as drops instead of incorporating these vitamins into the lard of diet 295 did not result in protection.

When in diet no. 295 the salt mixture was given at the 4 % level instead of 5 % no protection resulted. When, however, the salt mixture in diet 295 was replaced by the salt mixture of diet 330 (4 % level) a partial protection (50 %) was obtained.

The effect of copper sulfate, manganous sulfate, and potassium iodide added to the salt mixture in diet no. 295 in amounts corresponding to those in which they occur in the salt mixture of diet no. 330, was examined. It was found that the copper salt gave a partial protection.

Copper sulfate was then tested at 3 different levels, viz., 1/3, 1/1 and 3/1 times the amount corresponding to the salt mixture in diet no. 330. These experiments showed that the low concentration of copper did not protect, whereas the middle concentration gave a slight protection and the high concentration protected completely.

A repetition of this experiment showed a partial protective effect of copper for all three levels.

Although the effect of copper and the combined feature of diet 330 are interesting *per se*, it is not likely that they can account for the protective effect of fat-free hempseed, soybear

flour or a 30 % alcoholic extract of soybean flour. It is more likely that these materials contain a protective organic factor or factors insoluble in fat. This problem is being studied further.

## Summary.

A series of cereals, seeds and a type of yeast were tested for their ability to protect against gallstones in hamsters.

Soybeans and dried brewer's yeast were found to be particularly active. Hempseed was also found to be fairly active.

Hempseed was also active after extraction of the oil.

The oil and the ash of hempseed gave only a partial protection whereas the ether-extracted seed protected completely.

An extract of soybean flour prepared by boiling with 30 vol.% alcohol gave full protection. The same result was obtained with the extracted soybean flour.

When dried brewer's yeast was extracted with boiling water, the activity remained in the extracted yeast.

An artificial diet of another composition than the basal diet used in the experiments had very little tendency to produce gallstones in hamsters.

A certain (relatively high) amount of copper sulfate, added to the basal diet gave a partial protection against gallstones.

No protection was obtained by adding sitosterol, lecithins, inositol, cystine, taurine, and several other compounds and materials to the basal diet.

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# Schmerzimpulse bei Temperaturreizen.

Von

EBERHARD DODT.

Eingereicht am 30. Dezember 1953.

Die von HENSEL und ZOTTERMAN (1951 b), DODT und ZOTTERMAN (1952 a, b) und DODT (1952) an sensiblen Nervenfasern der Zungenschleimhaut der Katze durchgeführten Versuche haben die Beziehung zwischen Temperatur und Rezeptorentladungen für die spezifischen Temperaturendorgane in wesentlichen Punkten klargestellt. Im Gegensatz zu den älteren sinnesphysiologischen Vorstellungen ergaben die elektrophysiologischen Versuche für die Maximalaktivität der Temperaturfasern bei konstanter Hauttemperatur bei den Kaltrezeptoren relativ hohe und bei den Warmrezeptoren unerwartet niedrige Temperaturwerte. So zeigte die Mehrzahl der auf ihre Dauerentladungen untersuchten Warmrezeptoren ein Maximum um 40° C, wobei niedrigere Werte als 37° C niemals, höhere als 42° C nur in ganz seltenen Fällen beobachtet wurden. Spezifische Warmfaserimpulse waren bei Erwärmungen auf mehr als 45° C in der Regel nur bei plötzlichen Temperatursprüngen und auch hier nur in den ersten Sekunden zu sehen (DODT und ZOTTERMAN 1952 a). Aufgrund dieses Verhaltens, das sich bisher bei insgesamt 29 einzelnen Warmfasern bestätigen liess, sahen wir uns veranlasst, für die Hitzeempfindung bei Temperaturreizen, für die seit jeher eine Mischung verschiedener spezifischer Gefühlsqualitäten angenommen wurde, eine Beteiligung der Warmrezeptoren auszuschliessen. Es war demnach zu vermuten, dass die Hitzeempfindung durch eine Mischung von (paradoxer) Kälte- und Schmerzempfindung bedingt sei.

Für die spezifischen Kaltfasern wurden bei Zungentemperaturen oberhalb 45° C Dauerentladungen bereits sichergestellt (Dodt und Zotterman 1952 b), während über die bei Temperaturreizen auftretenden Schmerzimpulse bisher relativ wenig Quantitatives bekannt ist.

## Methode.

Die Operations-, Ableitungs- und Registriertechnik entspricht im wesentlichen jener vorangegangener Mitteilungen (Zotterman 1936, Hensel und Zotterman 1951 a). Zur Temperaturreizung der Zungenoberfläche wurde die von Hensel, Ström und Zotterman (1951) angegebene Thermode verwandt. Die Katzen wurden mit Chloralose-Urethan-Lösung (0.05 g Chloralose und 0.25 g Urethan in 7 ccm Ringer per kg Körpergewicht) anästhesiert; bei den Fröschen wurde unter Schonung der Reflexverbindungen vom N. glossopharyngeus zum N. hypoglossus das ZNS durch Ausbohren zerstört. Während die Versuche an der Katze durchweg an Einzelfaserpräparationen vom N. lingualis durchgeführt wurden, mussten wir uns beim Frosch im wesentlichen darauf beschränken, vom durchschnittenen N. glossopharyngeus nach Abstreifung des Epineuriums abzuleiten, da das Perineurium hier eine Nervenaufsplitterung mit Pincetten und Nadeln wie bei der Katze ohne Schädigung der Nervenfasern praktisch nicht zulässt. In einigen Fällen wurden die natürlichen Aufzweigungen des N. glossopharyngeus benutzt, die jedoch wegen der unmittelbaren Nähe zur Reizthermode und der Kleinheit des Tieres selten eine zur Ableitung genügende Länge besitzen, wobei die Ableitung ausserdem durch die ständige Schleimabsonderung der Mundhöhlenmucosa erheblich erschwert wird.

## Ergebnisse.

### I. Beobachtungen an sensiblen Nervenfasern der Katze.

Wenn wir in folgendem von »Schmerzimpulsen« sprechen, bedeutet diese Bezeichnung naturgemäss eine Interpretation. Die Gewissheit indessen, dass es sich bei diesen infrage stehenden Impulsen um solche in schmerzvermittelnden Fasern handelt, scheint uns durchaus nicht geringer als bei den Kälte-, Wärme- und Druckimpulsen und gründet sich — wie dort — auf einen Vergleich mit den subjektiven Empfindungen im sinnesphysiologischen Experiment am Menschen und ausserdem auf Erfahrungen, die der Untersucher mit Temperaturfasern des gleichen Nerven an insgesamt mehr als 200 Katzen machen konnte.

Bisherige Untersuchungen (Zotterman 1933, 1936, 1939) haben die Identifizierung von »schnellem« und »langsamem«

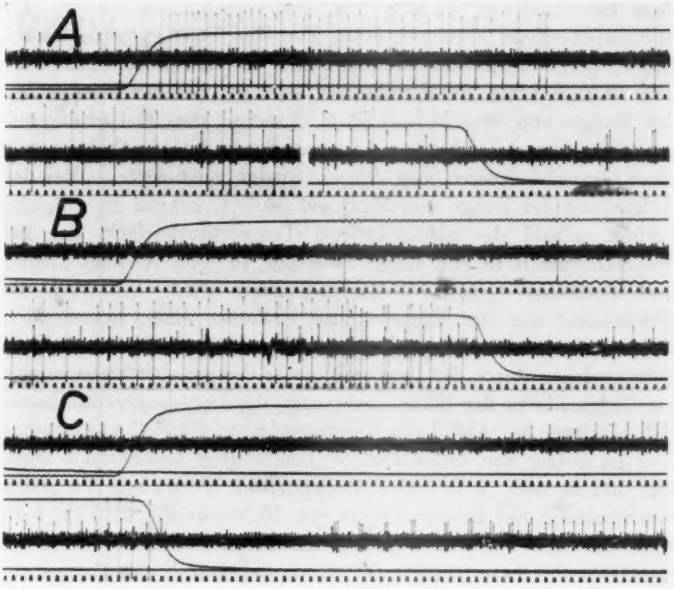


Abb. 1. Aktionspotentiale einer Nervenfaserpräparation aus dem N. lingualis der Katze bei thermischer Reizung der Zunge. Die Präparation enthält eine einzelne Kältefaser (kleine Spitzenpotentiale) und eine einzelne Schmerzfaser (grosse Spitzenpotentiale).

A 30.9°—55.4° C
B 30.6°—57.0° C
C 30.8°—60.3° C.

Anzeige der Temperaturänderung durch Galvanometer, am Anfang der Registrierung jeweils der Warmreiz, am Ende Wiederabkühlung. Die Photogramme sind von links nach rechts und von oben nach unten zu lesen. Zeitmarken 20 Hertz.

Schmerz mit verschiedenen Nervenfasertypen weitgehend gesichert: Der »first pain«, der sog. helle Schmerz, der in dünnen markhaltigen Fasern geleitet wird und der »second pain«, der sog. dumpfe Schmerz, dessen Übermittlung in marklosen C-Fasern erfolgt. Die im folgenden zu schildernden Beobachtungen, die bei Ableitung von Nervenfaserpräparationen des N. lingualis der Katze gemacht wurden, betreffen lediglich Schmerzimpulse von A-Fasern, da der N. trigeminus der Katze nach elektronenmikroskopischen Befunden von Gasser (Zotterman, persönliche Mitteilung) keine afferenten C-Fasern enthält.

Die in Abb. 1 wiedergegebenen Nervenimpulse, die von einer Präparation im N. lingualis abgeleitet wurden, stammen von

zwei verschiedenen Fasern, wie die verschiedene Grösse der Spitzenpotentiale erkennen lässt. Die eine Faser ist eine Kälte-Faser, deren Entladungsfrequenz bei der Ausgangstemperatur von 30.9° C etwa 10 Impulse/sec. beträgt; bei der Erwärmung der Zunge von 30.9° C auf 55.4° C stoppt ihre Entladung unmittelbar, um 4 sec. später als »paradoxe« Kälteentladung mit einer Impulsfrequenz von 9/sec. wieder einzusetzen. Bei der Abkühlung der Zunge von 55.4° auf 30.9° C kehren die Impulse zurück, sobald der aktivitätsfreie Temperaturbereich, der für die Kältefasern in der Regel zwischen 45°—38° C liegt (Dodt und Zotterman 1952 b), unterschritten wird. Während die Erwärmung auf die Kälteimpulse zunächst einen hemmenden Effekt ausübt, wird eine in der gleichen Präparation enthaltene Schmerzfaser durch die Erwärmung der Zungenoberfläche erregt. Im Gegensatz zu der Kältefaser stoppt die Impulsaktivität dieser Schmerzfaser bei Abfall der Temperatur auf 30.9° C unmittelbar und so lange, wie diese niedere Temperatur beibehalten wird. Bei den in Abb. 1 B und C dargestellten Verläufen, die gleich anschliessend bei Erwärmungen von 30.6° auf 57° C (Abb. 1 B) bzw. auf 60.3° C (Abb. 1 C) registriert wurden, ist zu erkennen, dass sich die Latenzzeiten für die paradoxen Kälteentladungen bei gleichzeitiger Erhöhung ihrer Impulsfrequenz mit den stärkeren Erwärmungen deutlich verkürzt haben, während für die Latenzzeit der Schmerzimpulse ein umgekehrtes Verhalten zu erkennen ist. Bei den Schmerzfasern ist also bei schneller Aufeinanderfolge der Reize trotz stärkerer Erwärmung nicht nur keine Zunahme, sondern im Gegenteil eine deutliche Erregbarkeitsminderung eingetreten. Zum Verständnis dieser merkwürdigen Aktivitätsabnahme ist zu sagen, dass die thermischen Reizungen der Zunge in Abb. 1 B und C ohne längeres vorheriges Verweilen auf einer tieferen Temperatur vorgenommen wurden. Der in Abb. 1 A zu beobachtende Verlauf war nach 10 Minuten dauernder Abkühlung ohne weiteres reproduzierbar.

Übereinstimmend mit den Befunden an spezifischen Kalt- und Warmfasern (Hensel und Zotterman, 1951 b, Dodt und Zotterman, 1952 a) sind auch bei Schmerzfasern bei konstanten Temperaturen Dauerentladungen zu beobachten; Abb. 2 zeigt als Beispiel die Impulsfrequenz von zwei einzelnen Schmerzfasern als Funktion der konstanten Zungentemperatur. Um allzu lange Erwärmungen der Zunge in diesen Temperaturbereichen zu vermeiden, wurde die Impulsfrequenz jeweils zwischen der

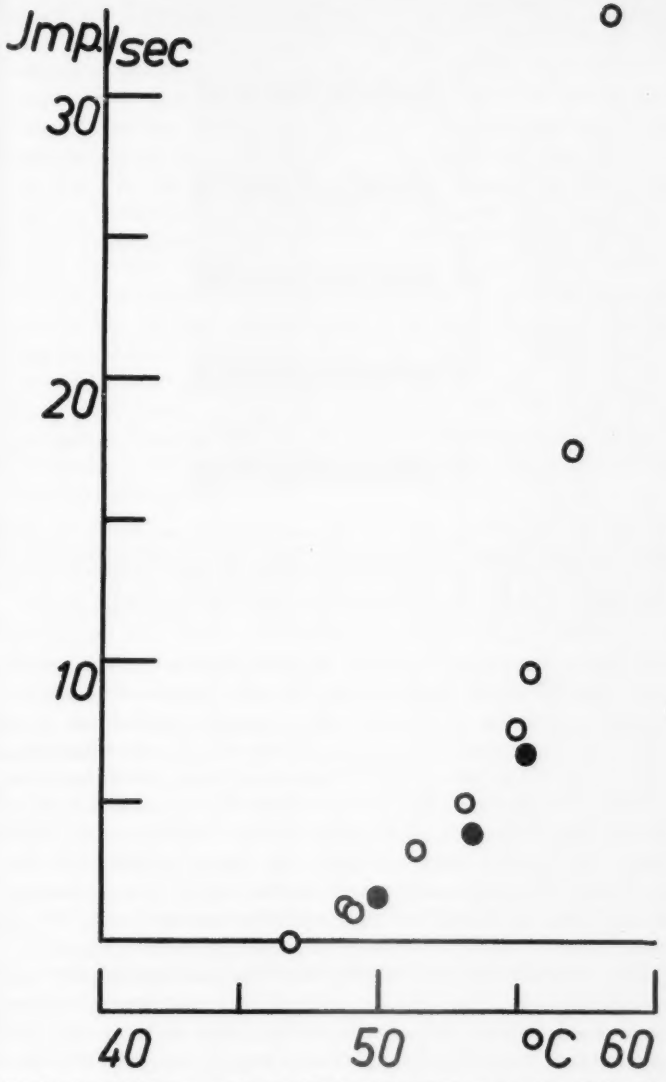


Abb. 2. Impulsfrequenz zweier spezifischer Schmerzfasern aus dem N. linguals der Katze als Funktion der Zungentemperatur.

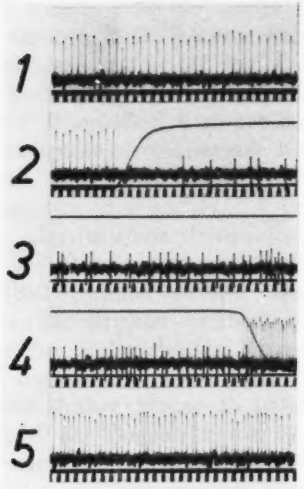


Abb. 3. Aktionspotentiale einer Nervenfaserpräparation aus dem N. lingualis der Katze bei thermischer Reizung der Zunge. Die Präparation enthält eine einzelne Berührungsfaser (grosse Spitzenpotentiale) und eine einzelne Schmerzfaser (kleine Spitzenpotentiale). Bei »2« Erwärmung der Zunge von 30°—54° C, bei »4« Abkühlung über den gleichen Temperaturbereich. Zeitmarken 20 Hertz.

15. und 20. sec. nach Beginn der Erwärmung bestimmt. Es ergibt sich dabei für die Schmerzimpulse übereinstimmend eine Schwellentemperatur von 47° C mit einem zunächst langsamen, dann schnellen Anstieg der Entladungsfrequenz, der bei einer Temperatur von 56° C bei der einzelnen Faser einen Wert von 33 Impulsen pro sec. erreicht. Daurentladungen dieser Fasern unterhalb 47° C wurden von uns nicht beobachtet. Damit erhalten die am Menschen gewonnenen Angaben über die Temperaturschwelle für den hellen Wärmeschmerz (knapp oberhalb 47° C: für die Zungenschleimhaut, v. UDRANSKY 1910; für die äussere Haut, VERESS 1902, SKOUBY 1951) ihre elektrophysiologische Bestätigung.

Zum Unterschied gegenüber den Wärmeimpulsen sind die beschriebenen Schmerzimpulse nicht nur bei Erwärmung, sondern auch durch Druck bequem auslösbar. Zum anderen sind die spezifischen Berührungs- und Druckfasern von den Schmerzfasern durch ihr Verhalten gegenüber Erwärmung deutlich verschieden: sie werden durch die Erwärmung entweder garnicht oder aber erst bei langdauernden Erhitzungen (eine Minute oder

mehr) auf Temperaturen oberhalb 55° C erregt (was praktisch ihrer Zerstörung gleichkommt) und zum anderen sind die Berührungsimpulse wesentlich grösser als jene der Schmerzfasern (ZOTTERMAN 1936, 1939). Niemals fanden sich bei thermischer Reizung bei den Berührungs- und Druckfasern Erregungsabläufe nach Art von Abb. 1. Ein Beispiel für die Wirkung einer Erwärmung auf die Tätigkeit einer spezifischen Berührungsfaser zeigt Abb. 3: Die durch konstanten Druck auf die Zunge hervorgerufenen Berührungsimpulse werden durch die plötzliche Erwärmung der Zunge von 30°—54° C unmittelbar und für die Dauer der Erwärmung gehemmt (ZOTTERMAN 1936); statt ihrer treten Impulse einer in der gleichen Präparation enthaltenen Schmerzfaser auf. Dass es sich bei dieser zweiten Faser um eine Schmerzfaser handelt und nicht um eine (paradoxerregte) Kaltfaser oder eine Warmfaser, geht daraus hervor, dass sich weder bei 30° C noch bei geringeren Temperaturen Dauerentladungen zeigen: bei Wiederabkühlung der Zunge von 54°—30° C sind lediglich wieder die Druckimpulse zu sehen.

## II. Beobachtungen an der Zungenschleimhaut des Frosches.

Im Anschluss an die Versuche bei der Katze war es interessant zu prüfen, ob auch bei poikilothermen Tieren eine periphere Thermorezeption stattfindet und in welcher Weise hier die Schmerzrezeption im Dienste der Thermorezeption steht. Die Schleimhaut der Froschzunge mit ihrer grossen Zahl freier Nervenendigungen zeigte sich hierbei als ein besonders günstiges Untersuchungsobjekt, zumal bei der geringen Körpertemperatur des Frosches bereits weit geringere Erwärmungen genügen als bei der Katze. Es war dennoch überraschend für uns, dass beim Frosch plötzliche Erwärmungen der Zunge um 3—4° bereits in Temperaturbereichen unterhalb 15° C zu deutlichen Aktivitätszunahmen im N. glossopharyngeus führen. Den Effekt einer schnellen Erwärmung der Froschzunge zeigt Abb. 4: etwa 0.25 sec. nach Beginn der plötzlichen Temperatursteigerung von 8°—24° C wird in der Ableitung vom N. glossopharyngeus eine kräftige Impulsaktivität sichtbar, die im Laufe der nächsten Sekunden langsam wieder zurückgeht (Abb. 4 A, untere Reihe). In einigen Versuchen wurde neben den Nervenimpulsen auf dem zweiten Strahl des Doppeloszillographen die Muskelaktivität der Mundöffner (M. depressor maxillae) der Gegenseite mitregistriert. Hier zeigt sich

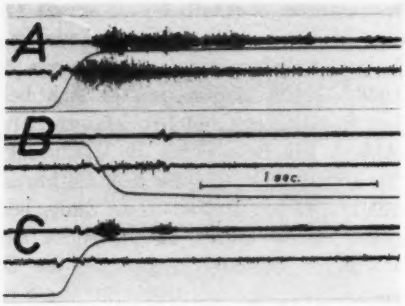


Abb. 4. Aktionspotentiale des N. glossopharyngeus (untere Reihe) und des M. depressor maxillae (obere Reihe) bei thermischer Reizung der Froschzunge.
A 8.2°—24.4° C
B 5 sec. später 24.4°—8.2° C
C sofort anschliessend an B 8.2°—24.4° C.

0.45 sec. nach Beginn der Erwärmung der Zunge eine anhaltende Muskelerregung (Abb. 4 A, obere Reihe), die den Nervenimpulsen somit im Abstand von etwa 0.2 sec. folgt und im übrigen eine recht gute zeitliche Parallelität zu den darunterstehenden Impulsen des N. glossopharyngeus ergibt. Der reflektorische Charakter dieser Muskelaktivität steht ausser Zweifel, da die Entladung nach beiderseitiger Durchschneidung der Nn. glossopharyngei ausbleibt.

Im Gegensatz zu der kräftigen Aktivität bei Erwärmung der Zunge ist bei plötzlicher Abkühlung weder in der Ableitung vom Muskel noch in jener vom Nerven ein Effekt zu erkennen (Abb. 4 B). Dieser Befund sowie der nocizeptive Charakter der Wärmeimpulse (»Zungen-Kiefer-Reflex«) lässt es fast sicher erscheinen, dass wir es bei den eben beschriebenen Erregungen kaum mit einer spezifischen Thermorezeptor-Aktivität zu tun haben. Hierfür spricht auch eine weitere Beobachtung: bei sogleich anschliessender Wiedererwärmung der Zunge ist bei Ableitung vom Nerven keine neue Aktivitätssteigerung sichtbar (Abb. 4 C). Wir haben somit ein entsprechendes Phaenomen vor uns wie bei den Schmerzfasern der Katze (siehe Abb. 1 B und C); wie dort ist auch beim Frosch nach der ersten Erwärmung der Zunge eine längere Erholungszeit notwendig, bevor ein neuer Schmerzreiz normale Erregbarkeit vorfindet. Diese Erregbarkeitsminderung als Ausdruck eines Wärmeblocks des peripheren Nerven anzusehen, kommt kaum in Betracht, da die Erscheinung auch bei einer

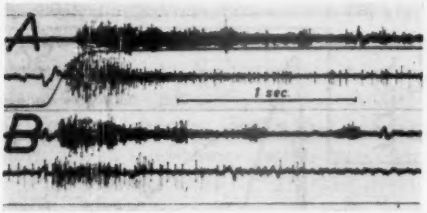


Abb. 5. Aktionspotentiale des N. glossopharyngeus (untere Reihe) und des M. depressor maxillae (obere Reihe). Bei (A) Erwärmung von 8.1°—32° C, bei (B) plötzlicher Druck auf die Froschzunge.

Endtemperatur zu beobachten ist, die mehr als 10° unter der Schwelle des Wärmeblocks der A-Fasern (BREMER und TITECA 1946) liegt.

Eine ähnliche Aktivität wie bei Erwärmung zeigt sich im N. glossopharyngeus bei kräftigem, plötzlichen Druck auf die Froschzunge. Auch hier folgt den Nervenimpulsen (untere Reihe von Abb. 5 B) im geringen zeitlichen Abstand (0.1 sec.) eine reflektorische Muskelentladung (siehe darüberstehendes Potential von Abb. 5 B). Die Frage, ob diese nocizeptiven Druckimpulse mit jenen bei Erwärmung identisch sind, zeigen unsere Befunde nicht ohne weiteres. Doch dürfte dies sehr wahrscheinlich der Fall sein, da TASAKI und Mitarbeiter (1952) von anatomischen Einzelfaserpräparationen der Kröte (myelinisierte Hautnervenfasern, 3—5 $\mu$ Dicke) sowohl durch Nadelstiche wie durch Warmreizung des zugehörigen Hautareals Impulse erhielten.

Eine sichere Entscheidung darüber, ob die bei Erwärmung der Froschzunge auftretenden nocizeptiven Impulse auch als Dauerentladungen bei konstanter Temperatur zu beobachten sind oder nicht, ist bei den oft zu beobachtenden »Spontanentladungen« des N. glossopharyngeus recht schwierig und kann nur durch Experimente an anatomisch isolierten Fasern gefällt werden. In unseren Experimenten haben wir den Eindruck gewonnen, dass diese Erregung eher phasisch als nach Art einer Dauerentladung verläuft, da bereits zwei Sekunden nach Beginn der Erwärmung nurmehr ein verschwindend kleiner Teil der Faser aktiv ist (s. Abb. 4 A).

## Diskussion.

Die Vorstellung, dass die Hitzeempfindung bei Temperaturreizen duch eine gleichzeitige Erregung von Kälte- und Schmerz-

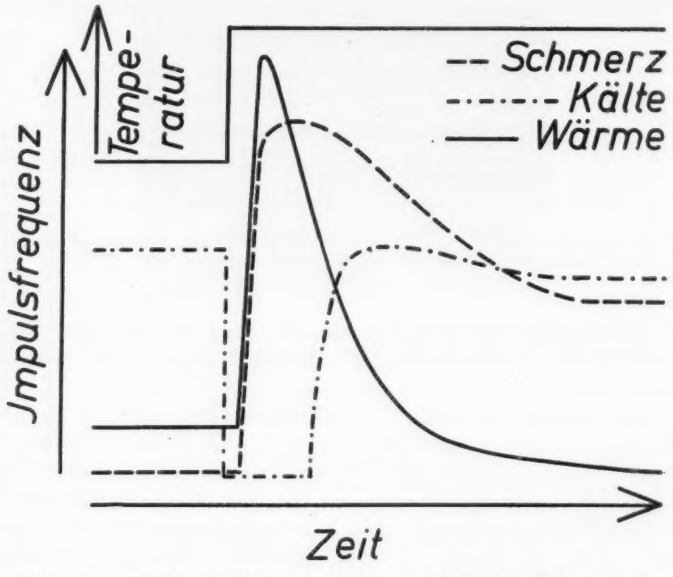


Abb. 6. Schematische Darstellung der Änderung der Entladungsfrequenz einer einzelnen spezifischen Schmerz-, Kälte- und Wärmefaser aus dem N. lingualis der Katze vor, während und nach schneller Erwärmung der Zunge von 30°—55°C.

fasern (paradoxe Kälte und Schmerz) zustandekommt (DODT und ZOTTERMAN 1952 a), wird durch die vorliegenden Beobachtungen nun auch für die Schmerzimpulse gesichert. Abbildung 6 zeigt schematisch das Verhalten von Schmerz-, Wärme- und Kältefasern bei einer plötzlichen Steigerung der Zungentemperatur von 30°—55° C: Der einleitende Vorgang bei der Temperaturänderung ist eine kräftige Erregung der Warmfasern, die jedoch bereits nach wenigen Sekunden völlig erlischt. Gleichzeitig mit der Warmfaseraktivierung oder wenige Sekundenbruchteile später kommt die Erregung der Schmerzfaser hinzu, wobei die Impulsfrequenz im Augenblick der Temperatursteigerung zwar wesentlich geringere Werte als bei den Warmfasern ergibt, jedoch — da sie Dauerentladungen zeigt — für die Aufrechterhaltung des Hitzegefühls von Bedeutung sein muss. Das gleiche gilt für die »paradoxen« Kaltfaserimpulse, die bei der Temperatursteigerung eine »Überschiessende Erregung« fast völlig vermissen

lassen, jedoch gleich den Schmerzfasern auch bei konstanter Temperatur erhebliche Daueraktivität zeigen.

Unsere Beobachtungen am N. lingualis der Katze geben erste Anhaltspunkte über die Aktivität spezifischer Schmerzfasern bei definierten Temperaturreizen. Gegenüber den Kälte-, Wärme- und Druckfasern zeigen die Schmerzfasern zwei charakteristische Unterschiede: 1) ihre unspezifische Erregbarkeit durch Erwärmung und Druck, 2) die Höhe der Spitzenpotentiale, welche im Vergleich zu jener anderer Fasern Rückschlüsse auf das Kaliber der Fasern erlaubt.

Die unspezifische Erregbarkeit der Schmerzfasern war in unseren Versuchen das wichtigste Kriterium zur Differenzierung von anderen Fasern. Zwar sind Druckfasern und Warmfasern ebenfalls »inadäquat« durch thermische Reizung der Haut erregbar (Hensel und Zotterman 1951 c, Dodt und Zotterman 1952 a), doch zeigt die dabei auftretende Erregung einmal rein phasischen Verlauf (keine Dauerentladungen) und zum andern findet eine solche Erregung nur bei Abkühlung statt. Ausser den Schmerzfasern sind uns jedenfalls keine Nervenfasern bekannt, die sowohl bei Erwärmung wie bei Druck auf die Haut Dauerentladungen zeigen. Selbst bei Druck oder plötzlicher Abkühlung des Nerven, wobei sensible A-Fasern jeder Art erregt werden (Dodt 1953), sind lediglich phasische Erregungen zu sehen.

Aus den Untersuchungen Zotterman's (1936, 1939) geht hervor, dass die myelinisierten Schmerzfasern ihrem Kaliber nach der $\delta$-Gruppe zuzurechnen sind. Auch Tower (1943) fand bei Ableitung von Einzelfaserpräparationen des N. ciliaris longus der Katze, dass die bei mechanischer Reizung der Cornea erregten Nervenfasern eine Dicke von 7 $\mu$ nicht überschreiten. Unsere Versuche bestätigen diese Beobachtungen insofern, als die Spitzenpotentiale unserer Schmerzfasern deutlich grösser sind als jene der spezifischen Kaltfasern (1.5—3 $\mu$, mikroskopische Messung von Maruhashi, Mizuguchi und Tasaki 1952) und wesentlich kleiner als jene der spezifischen Druckfasern (8—15 $\mu$).

Die beobachtete Erregbarkeitsabnahme der Schmerzfasern bei wiederholten Erwärmungen der Zunge steht in Übereinstimmung mit den Erfahrungen des täglichen Lebens und den Angaben der Literatur (Stein und v. Weizäcker 1927), wonach sich bei wiederholten Schmerzreizen ein allmähliches Anwachsen der Reaktionszeiten und eine Abnahme der Empfindungsintensität einstellt. Die lange Erholungszeit, die im Anschluss an die erste

Erwärmung erforderlich ist, um wieder normale Erregbarkeitswerte zu erhalten, wird unter der Annahme verständlich, dass die Schmerzerregung durch Anreicherung von Stoffwechselprodukten einer vorausgehenden Gewebsschädigung eingeleitet wird (v. FREY 1894). Wir können heute aufgrund der schmerzerzeugenden Wirkung von intrakutan injiziertem Histamin, Acetylcholin und Kaliumchlorid (EMMELIN und FELDBERG 1947; SKOUBY 1951, 1953) annehmen, dass der chemische Reiz den adäquaten Reiz für die Schmerzrezeption darstellt.

Die Versuche beim Frosch zeigen uns, dass das Vorkommen von Thermorezeptoren im engeren Sinne bei poikilothermen Tieren von Spezies zu Spezies verschieden ist. Wenn wir die von SAND (1938) an Raja bei thermischer Reizung der Lorenzinischen Ampullen an Einzelfasern des N. facialis beobachteten Impulse als Erregungen spezifischer Kältefasern ansehen, so müssen wir andererseits für die Froschzunge das Vorkommen peripherer Kältesinnesorgane eindeutig verneinen. Bei der ausgeprägten reflektorischen Wirkung der Warmreize glauben wir vielmehr, dass es sich bei den beobachteten Impulsen im N. glossopharyngeus um nocizeptive Erregungen handelt. Die Parallelität der reflektorischen Muskelaktivität mit den afferenten Impulsen weist darauf hin, dass diese Reflexaktivität durch die in dünnen markhaltigen Fasern geleiteten Impulse zustandekommt (siehe auch ZOTTERMAN 1933). Inwieweit die von ZOTTERMAN für die Schmerzerregung im allgemeinen sichergestellte Beteiligung von C-Fasern auch beim Wärmeschmerz eine Rolle spielt, zeigen unsere Versuche nicht; wahrscheinlich sind die in marklosen Fasern auftretenden Potentialschwankungen zu gering, um bei Ableitung von einem Nerven von der Dicke des N. glossopharyngeus sichtbar zu werden — falls dieser Nerv C-Fasern besitzt.

## Zusammenfassung.

Die im Anschluss an Versuche über das Verhalten spezifischer Wärme- und Kältefasern gegenüber Temperaturreizen entwickelte Vorstellung, dass die Hitzeempfindung bei äusseren Temperaturreizen durch eine gleichzeitige Erregung von Kälte- und Schmerzfasern (»paradoxe« Kälte und Schmerz) zustandekommt, wurde durch Aktionsstromuntersuchungen an spezifischen Schmerzfasern der Katzenzunge bestätigt. Danach werden durch Temperaturreize von mehr als 47° C Entladungen kleinkalibriger mark-

haltiger A-Fasern hervorgerufen, die sowohl als Dauerentladungen (bei konstanter Temperatur) wie auch (bei plötzlicher Erwärmung) als frequente Impulsstösse auftreten. Bei wiederholten und starken Erwärmungen kann die bei plötzlicher Erwärmung auftretende Impulssteigerung für mehrere Minuten eine starke Abschwächung zeigen. Im Gegensatz zu den spezifischen Warmfasern sind diese Schmerzimpulse sowohl durch Erwärmung als auch durch Druck auslösbar, während die Schmerzfasern von den spezifischen Druck- und Berührungsimpulsen durch ihre Erregbarkeit gegenüber Warmreizen (hier tritt bei Druckfasern Wärmelähmung ein) unterschieden sind. Aufgrund eines Vergleichs der Spitzenpotentiale der Schmerzfasern mit jenen der Kalt-, Warm- und Druckfasern wird für die beobachteten Schmerzfasern eine Dicke von 3—7 $\mu$ angenommen.

Bei thermischer Reizung der Froschzunge sind bei plötzlichen Erwärmungen um mehr als 3° Impulse von N. glossopharyngeus in ähnlich dimensionierten Nervenfasern wie vom N. lingualis der Katze ableitbar. Im Gegensatz zur Katze sind diese Impulse bereits in Temperaturbereichen unterhalb 15° C auslösbar. Sie treten bei Erwärmung, nicht aber bei Abkühlung auf. Bei schnell wiederholter Erwärmung kann der Effekt auch beim Frosch bis zum völligen Fehlen vermindert sein; längeres Verweilen auf Temperaturen oberhalb 33° C führt zum Ausbleiben jeden Effektes. Bleibt der N. glossopharyngeus einer Seite intakt, so führen die Warmreize nach einer Überleitungszeit von rund 0.2 sec. zu einer z. B. vom M. depressor maxillae ableitbaren kräftigen reflektorischen Muskelaktivität, die ausgesprochene Parallelität zu den afferenten Impulsen im N. glossopharyngeus erkennen lässt. Der gleiche Effekt ist sowohl afferent wie reflektorisch statt durch Erwärmung durch Druck auf die Zunge auslösbar. Die ausgeprägte reflektorische Wirkung der Warmreize und das Ausbleiben der Erregungen bei Abkühlung sprechen dafür, dass es sich bei diesen afferenten Impulsen um nocizeptive Erregungen handelt.

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